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# FUNCTIONAL ORGANIZATION OF THE SPINAL CORD

DAVID P C LLOYD[1]

*Laboratories of The Rockefeller Institute for Medical Research New York*

The last decade has witnessed a major change in experimental approach to the problems of spinal cord function Investigation has turned from myographic analysis of the reactions of peripheral effectors to the direct study, by electronic methods, of the spinal cord itself and its peripheral nerve paths Attention has been directed to the fundamental properties of neurons and of synaptic regions, and also to the functional architecture of the spinal mechanism as it appears when played upon by various systems of reflex and higher control

Recent developments in the study of nervous organization owe their measure of success to precise timing of events made possible by high speed, high fidelity recording methods, and to precise spatial localization of activity made possible by the development of refined microelectrode technique

The central nervous system consists of primary afferent fibers, which transmit impulses from the periphery, motoneurons, which transmit impulses to the periphery, and interneurons connected in complex patterns with the afferent neurons and motoneurons Potentially, activity entering the central nervous system by any given neuron path is capable of influencing by more or less devious routes every other neuron therein In practice one finds a rather high degree of "local sign" attending reaction within the nervous system, a fact exemplified by the cortical response to tactile stimulation at a given peripheral locus (2), or the precise fractionation of the intrinsic spinal mechanism by various descending systems (36, 38) In addition to the spatial relations of a given response there are characteristic temporal relations between a stimulus and its action at a given point. An accurate accounting for the overall latent period for a given response is essential for an understanding of the pathway through which the response is mediated

Synaptic and nuclear delay At first sight pools of neurons seemingly behave in a fundamentally different manner when activated by synchronous and asynchronous presynaptic activity In the former case Lorente de Nó (47, 48) and others have shown that transmission occurs with a relatively fixed synaptic delay and total latency equals in time the sum of conduction time and synaptic delays It is only in the simplest paths that such a reckoning represents the true state of affairs A much longer delay is encountered at synaptic regions when activation results from a statistically smooth waxing and waning barrage of impulses This longer time interval may be designated nuclear delay (36, 38) A clear understanding of the definitions and implications of the terms synaptic delay and nuclear delay is a useful preliminary for discussion of the problems of neural organization

[1] Now at the Laboratory of Physiology, Yale University School of Medicine New Haven Connecticut

The concept of synaptic delay introduced by Sherrington was quite adequate to, and in fact did, cover both types of delay recognized by contemporary description When Lorente de Nó revealed the relatively fixed period of time lost by virtue of transmission through a single synaptic region under conditions of near-ideal synchronization, a new concept of synaptic delay was introduced At the present time, and from a purely operational standpoint, synaptic delay is defined as the difference in time between transmission over a pathway of given length involving a synaptic relay, and transmission in a simple axon of equal length that has the properties of the parent presynaptic fiber for an equivalent length, and those of the postsynaptic fiber thereafter (cf 57, p 262) The minimum synaptic delay so defined in several alpha-tempo systems amounts to 0 5 to 1 0 msec Such an interval of time is, however, a formal synaptic delay and includes time devoted to 'extrasynaptic' events, conspicuously conduction in the fine terminal branches and endings From a theoretical point of view synaptic delay may be defined as the time interval between the arrival at the synapse of the last presynaptic impulse necessary to secure transmission and the onset of the resulting postsynaptic action Once a full accounting is made for all the factors contributing to the so-called minimum synaptic delay it seems possible that synaptic time in the strictest sense will prove vanishingly small

Frequently impulses from a given source may impinge upon a motor nucleus for several milliseconds before motor discharge results Thus following stimulation of the brachial plexus excitatory impulses enter the motoneuron pools of the lumbar enlargement 2 to 3 msec before the motoneurons discharge During this interval the excitation of the motoneurons is subliminal, but progressively greater, as tested by direct (two-neuron-arc) reflex excitation, until discharge finally occurs Since the term synaptic delay in current concepts is descriptive only of the relatively fixed time interval discussed above, it can no longer be used in the older sense to include the period of increasing excitation leading to discharge (cf 15) For this period the term nuclear delay has been introduced (38) Nuclear delay may be defined as the time interval between the arrival at the synapse of the first presynaptic impulse to the onset of postsynaptic action Under the condition of ideal synchronization of a given presynaptic volley it is clear that synaptic and nuclear delay would have identical values

Since there is good reason to believe that successive impulses in the same presynaptic element are incapable of summation (46), nuclear delay is to be considered as a manifestation of the combined activity of a number of presynaptic units asynchronously active Even so, by rotation of elements, repetition of presynaptic action may contribute to the rising level of excitability over a period of time

The concept of nuclear delay is of the greatest importance in studies on functional organization since the arrival of the first impulses at a given point rather than the arrival of the last impulses defines the 'characteristic' pathway to that point from the site of origin of the transmitted activity In short, central latency is equal to the sum of conduction time and nuclear delays (38)

THE LOCAL SPINAL MECHANISM Although it is frequently regarded as such,

the local spinal mechanism is not strictly speaking a segmental structure, for the reflex collaterals from any given dorsal root ramify over a number of neighboring segments Then too the interneurons supplied by a given dorsal root reach out to the white columns to embrace together a number of segments (7, 62) The reflex discharge through this mechanism, obtained by single shock stimulation of a dorsal root while recording from the ventral root of the same segment or a neighboring segment, displays a prominent initial peak, which is transmitted through arcs of two-neurons, i e , directly from primary afferent fiber to motoneuron (13, 54) This peak is followed by an irregular discharge elevation of some 10 msec duration, which is transmitted through multineuron arc pathways The two-neuron-arc reflex is more circumscribed than is the multineuron arc reflex (41) which lends the additional weight of functional evidence to support the concept of circumscribed and diffuse reflex mechanisms proposed by Cajal

It is impossible from ventral root recordings to know the peripheral distribution of the several components of the reflex discharge through the local spinal mechanism So too, on stimulation of a dorsal root, fibers of many sensory modalities are activated together in unnatural combination Therefore, the reflex discharges so obtained and recorded are of anatomical rather than of functional significance, they contain the elements of a number of ipsilateral reflexes, artificially admixed One of the major problems in spinal organization has been the resolving of the local spinal reflex discharge into its functional components

Associated with activation of the local spinal mechanism are 'slow' potentials which may be recorded by means of electrodes placed on or within the spinal cord, or by means of electrodes placed in close proximity to the cord on its associated dorsal and ventral roots The analysis of these slow potentials provides one of the most difficult problems of neurophysiology and one which, on the basis of published work, has been accorded little critical attention From the standpoint of the problems under discussion, experience has shown that the slow potential signs of activity are so widespread and so little understood as to be of little use in locating the active elements under any given conditions of excitation Fortunately the use of refined microelectrode technique has never failed to reveal the presence of localized discrete spike potentials during the course of induced activity, even in the cerebral cortex where the existence of discharges of spike dimensions is rarely recognized

The constitution of afferent nerves The dorsal roots contain fibers varying from approximately 20 micra to 1 micron in diameter (61) It has been found that only the largest of these, from 20 to 12 or 13$\mu$, have direct connection with the motoneurons (39) The medium and small fibers of the dorsal roots contribute exclusively to the multineuron-arc reflex discharges In the peripheral nerves major segregations of fibers occur, and these are of great reflex significance. The largest fibers are found only among the afferent fibers from muscle (61, 14, 52) In harmony with these histological observations is the fact that two-neuron arc reflex discharges are provoked by stimulation of muscle afferent fibers and not by the stimulation of cutaneous nerves (39, 41) Medium size fibers (12–6$\mu$) are found most prominently in cutaneous nerves (61, 14, 21, 53)

but are also present in muscle nerves The finer myelinated fibers, those usually spoken of as delta fibers, again are to be found in both muscle and cutaneous nerves, being more prominent in the latter Another group, consisting of unmyelinated fibers, may be included, for these are of proven reflex function (8, 4) The unmyelinated fibers form an impressive fraction of the total fiber count of a sensory nerve

THE MYOTATIC REFLEX Evidence has now accumulated to show that the reflex response to stretch is mediated through arcs of two neurons (39, 41, 42) One important characteristic of the myotatic reflex is that it appears only in the muscle or part of a muscle subjected to stretch (66, 33) Likewise it is a characteristic of the two-neuron-arc reflex that it appears only in the peripheral muscle nerve subjected to stimulation, it does not appear in other motor nerves nor in the nerve to one head of a muscle, the nerve to the other head of which is stimulated (41) Other experiments utilizing natural stimulation (42) have shown that the afferent response to stretch is transmitted in fibers of high conduction velocity (116 M per sec ) and therefore of large diameter Furthermore, it has been shown that the reflex response to sudden stretch is transmitted with but a single synaptic delay The demonstration that a reflex evoked by a natural adequate stimulus is transmitted with no more central delay than that required for transmission of the segmental two-neuron-arc reflex resulting from dorsal root stimulation should dispose finally of any fears that the two-neuron-arc reflex is by nature an artefact, or that excitatory events other than the detonator action of impinging afferent impulses must antecede normal reflex transmission

Two-neuron-arc reflex discharges are to be found also in association with flexor muscle pathways The conditions for recording such discharges are precisely those obtaining in the case of extensor muscle pathways (41) The reflex represented by such activity is best known as the 'pluck' reflex or flexor tendon-jerk (1)

THE FLEXOR REFLEX While the reflex effect attending stimulation of the low threshold muscle afferent fibers is confined to two-neuron-arc pathways, the reflex discharge following stimulation of the medium and small myelinated fibers of either cutaneous or muscle nerves has all the attributes of the multi-neuron-arc discharge as encountered in the segmental reflex In distribution the resulting reflex discharge is largely restricted to the motor nerves of flexor muscles, and represents the flexor reflex The shortest pathway for transmission of this reflex discharge involves an intercalated neuron between the primary afferent fibers and the motoneurons (41) Until recently it has been supposed that direct connections between afferent fibers and motoneurons serve flexor reflexes as well as stretch reflexes In consequence the functional significance of the circumscribed and diffuse reflex mechanisms as conceived by Cajal from histological evidence has been overlooked It is now clear that the direct and restricted two-neuron-arc pathway (the circumscribed reflex of Cajal) serves to limit to the muscle stretched the excitatory reflex field of action of the afferent influx from tension receptors In contrast, the interneuron intercalated in the minimum flexor reflex pathways provides the structural mechanism of insuring the diffusion of the flexor reflex to many or all the synergically acting muscles (the diffuse reflex of Cajal)

One of the most interesting problems awaiting solution is that of relating the sensory modalities and reflex effect. It appears that even the largest fibers in cutaneous nerves, when stimulated, contribute to the flexor reflex, as do the smaller fibers of the delta group. The flexor reflex is customarily regarded as the response to painful stimulation, although final justification for a specific relationship is not yet available. There seems to be general agreement that delta fibers carry pain producing impulses (cf 19), but Zotterman's experiments with touch stimulation (75) indicate that delta fibers carry touch as well as pain. If all the delta fibers contribute to the delta flexor reflex, it follows that touch as well as pain can promote flexion of the limb, but there is no certain evidence that this is the case.

Again, in summarizing the experimental evidence relative to pain fibers, Gasser (19) finds indications that there must be some fairly large-sized pain fibers. Such fibers may account for the flexor reflex obtained by weak stimulation of cutaneous nerves, but since the range of fibers above delta accommodates temperature and pressure fibers, it is impossible to say what modalities are represented in a stimulated volley relayed from afferent fibers into the flexor reflex connections. A partial solution to these problems might be forthcoming if it were known to what extent the medium and small myelinated afferent fibers, by collaterals, feed into the local reflex mechanism.

Similar problems attend the correlation of sensory modalities represented by the medium and small afferent fibers arising in muscle with the reflex effect of stimulating these fibers (flexion). Presumably some of these fibers in certain situations are concerned with the mediation of the lengthening reaction.

THE EXTENSOR THRUST AND REFLEXES OF CROSSED EXTENSION. At the present time there is little systematic knowledge of the central mechanism of these two reflexes. It is known from the work of Sherrington (62, 63, 64, 66), that the afferent pathway for the extensor thrust reflex is contained in the plantar nerves, but stimulation of these nerves evokes only flexion (66) and discharges into flexor nerves. There is good reason for stating that the extensor thrust (41) and the crossed extensor reflex (50) are both transmitted through multi neuron-arc pathways, but certain knowledge of the minimum pathway and relay points is not as yet available. In the case of the extensor thrust it would seem that this knowledge will only be obtained by the use of natural stimulation.

ACTIVITY IN LONG SPINAL REFLEX PATHWAYS. Reflex action from forelimb to hindlimb is mediated conspicuously by the long spinal fibers described by Sherrington and Laslett (67). In chronic spinal animals the intrinsic mechanisms of the spinal cord may be studied in isolation from the extrinsic mechanisms by virtue of degeneration of the latter. Direct stimulation of the ventrolateral tracts in such preparations shows that the long spinal fibers of the ventrolateral columns conduct impulses at alpha velocity, and that these impulses are relayed into the short spinal fibers of the ventrolateral columns (43). The relationship of long spinal fibers and short spinal fibers, then, is similar to the relationship between supraspinal (bulbospinal) fibers and short spinal fibers (34, cf below). Additional information as to the activity of the spinal system has been gained by reflex activation of the system rather than by direct stimulation of the tracts

themselves (38) When activated reflexly, the discharges descending through the spinal system possess the diffuse waxing and waning nature of nuclear discharge in contrast to the synchronized volley resulting from direct stimulation

Aboral transmission of the activity evoked by reflex stimulation on one side of the body involves intrinsic spinal tracts of both halves of the spinal cord, indicating a free decussation within the brachial field of the cord Activity eventually reaches motoneurons of the lumbar region by strictly ipsilateral paths, by crossed paths, and by the recrossing in the lumbar region of crossed activity, due to the free yoking of the halves of the cord in the lumbar enlargement The lateral columns are concerned with strictly unilateral transmission The ventral columns subserve bilateral as well as unilateral transmission The dorsal columns appear not to be of primary importance

Internuncial activity, resulting in the lumbar enlargement from afferent volleys to the cervical enlargement, is confined to the ventral horn Conspicuous among the active neurons are those constituting the nucleus of the anterior commissure, which fact is in harmony with the observed degree of importance to be attached to crossed conduction of long spinal reflex activity

Some motoneurons of the lumbar enlargement are inhibited at the calculated time of arrival of the first long spinal impulses at the site of action With the onset of internuncial activity in the ventral horn of the lumbar enlargement certain two-neuron-arc reflexes are facilitated and, after a few milliseconds nuclear delay, motoneurons begin to discharge in response to the long spinal reflex excitation

The Schiff-Sherrington phenomenon provides an interesting and somewhat perplexing instance of long spinal action in the ascending sense (59)

Ascending projection systems Many papers are to be found dealing with the cortical response, cerebral and cerebellar, to stimulation of peripheral nerves, or to natural stimulation, superficial or deep, but only in a very few cases has any attention been accorded the spinal mechanism mediating the cortical response

*The dorsal columns* The mean maximal conduction rate of impulses ascending through the dorsal columns approximates 70 M per sec (25) Since many afferent fibers conduct impulses at rates approaching 120 M per sec (42) either a selection of neurons or a change in conduction properties occurs soon after the dorsal root fibers reach the posterior columns Gasser and Graham (20) described the slowing of velocity taking place within a few centimeters of the root entry, and attributed it to decrease in diameter of the axons after giving off collaterals to the gray substance While this is undoubtedly the case, it is well to bear in mind the possibility that the dorsal columns contain significant selections from among the fibers of the dorsal roots, the largest fibers perhaps extending only far enough to plunge in to the Clarke-Stilling nuclei

The long tracts of the dorsal columns terminate in the nucleus gracilis and nucleus cuneatus The outstanding feature of transmission through the nucleus cuneatus is the powerful 'one-to-one' relay to the medial lemniscus (70) Activity of more complex origin is also present in the medial lemniscus, part of which may be ascribed to the ascending homologue of the dorsal root reflex described by Hursh (30) All except the initial relay is easily inhibited by repetitive activa-

tion, with the end result that transmission of a tetanic series through the nucleus cuneatus takes the form of a series of synchronized 'jets' into the medial lemniscus

*The dorsal spino-cerebellar (Flechsig's) system* According to the analysis of Grundfest and Campbell (25) the origin of Flechsig's tract in the column of Clarke receives further confirmation The tract fibers reach out from the column of Clarke to the dorso-lateral surface of the lateral column and ascend to the medulla as a flat ribbon of fibers For further detail of the fine structure of this tract the important paper of Sherrington and Laslett (68) should be consulted. Grundfest and Campbell (25) find that Flechsig's tract is activated by stimulation of muscle afferent fibers, but not by stimulation of skin afferent fibers This fact would suggest that the large afferent fibers which are responsible for transmission of the stretch reflex (41, 42) and the direct inhibitory actions of dorsal root fibers (39) also supply the cells of Clarke's column There seems to be no reason to suppose that distinct afferent fibers supply the stretch reflex and the proprioceptive projection to the cerebellum, and in all probability by collateral connection Flechsig's tract is activated *pari passu* with stretch reflexes

It is important to recall the observation of Schäfer (60) that lesions involving the pyramidal tract (and presumably other descending fibers) result in the appearance of large numbers of degenerated fibers "passing from the pyramidal tract (below the lesion) towards Clarke's column, curving round the ventral aspect of the section of that column and losing themselves as fine (probably branching) fibres amongst its cells" In general, Schäfer's viewpoint is so strikingly confirmed by recent functional studies on the pyramidal tract (36), that one must consider the interesting possibility that higher (i e., descending) systems which influence the tendon jerks may also influence the ascending concomitant of the tendon jerk at its point of relay in Clarke's column. The possibility would appear to merit careful investigation

Impulses in Flechsig's tract are conducted at high velocities by large fibers (cf 25, 26), with the result that they soon outstrip dorsal column impulses evoked in parallel, but not similarly subjected to synaptic relay with consequent loss of time (25) The earliest components of the cerebellar response to stimulation of a mixed nerve (tibial) are transmitted through Flechsig's tract for section of it blocks them The late component of the cerebellar response, in contrast is not so blocked, nor is the (late) response provoked by stimulation of the purely cutaneous saphenous nerve Presumably the late component resulting from stimulation of the tibial nerve is the result of stimulation of the cutaneous afferent fibers in that nerve In this connection it is of interest that a number of workers (11, 25, 12, 69) have recently reported cerebellar responses to stimulation of skin nerves or to natural tactile stimulation. Grundfest and Campbell's results show that the cerebellar response to stimulation of cutaneous afferent fibers is mediated by some other than Flechsig's tract

*Other ascending systems* Little is known, or may be conjectured as to the functional aspects of other ascending systems of the spinal cord. Many such systems consist of fine fibers, which places difficulties in the path of investigation

In a recent paper, Cooper and Sherrington (9) describe chromatolysis of large

cells scattered along the margin of the ventral horn following contralateral hemisection in the cervical or thoracic cord Tower, Bodian and Howe (73) likewise found chromatolysis of the 'spinal border cells' after isolation of a segment of the spinal cord According to the evidence of Cooper and Sherrington the spinal border cells give rise to an ascending decussating tract which they interpret to be Gower's tract (ventral or indirect spino-cerebellar tract) Since Cooper and Sherrington find some chromatolysis of the crossed Clarke's column it is still possible that this column contributes a part of the inflow to the tract of Gower

DESCENDING PROJECTION SYSTEMS *The ventrolateral descending tracts* The projection through the dorsal longitudinal bundle to the ventrolateral columns of the cord derives largely from the vestibular and reticular nuclei The major influences mediated through this system, conveniently termed the bulbospinal correlation system, are vestibular (and cerebellar) in origin

The tract fibers of the bulbospinal correlation system are rather uniform in size and possess a high conduction velocity, as judged by the absence of dispersion in a volley of impulses conducted from the dorsal longitudinal bundle to the sacral cord, a distance of some 30 centimeters (34) The bulbospinal fibers when activated in isolation have relatively little direct action upon the motoneurons, but do exert a powerful driving force upon the large fiber short propriospinal or intrinsic spinal neurons of the ventral horn Impulses from the long tracts are relayed, apparently at all levels into the ventral horn interneurons (short spinal neurons) which in turn play powerfully upon the motoneurons Backing up this powerful three-neuron pathway from the medulla to the periphery is a wealth of asynchronous internuncial discharge, probably confined to the ventral horn, which by "reverberating" onto the short spinal nuclei, provides for the observed powerful facilitation of those nuclei with the advent of repetitive stimulation

Motoneuron discharge resulting from the convergence of tract volleys initiated in the bulbospinal system and local internuncial activity resulting from similar volleys is controlled in time by the action of the short spinal neurons On the other hand, when the same tract volleys converge with internuncial activity derived from primary afferent volleys, all other conditions being equal, motoneuron discharge advances from the control of the short spinal neurons to that of the primary tract fibers, and the direct functional pathway from the tract fibers to the motoneurons supersedes the indirect pathway through the short spinal neurons The available evidence unfortunately does not permit one to say whether the motoneurons controlled by the direct tract action and those controlled through the short spinal fibers supply muscle of the same or of opposed action The significance of the experimental finding accordingly is obscure If the motoneurons discharged in each case are of different, and perhaps reciprocal, function, then it seems probable that the actions just described provide an essential mechanism for the production of rhythmic alternating activity continuously under the control of a single projection system

*The pyramidal system* The pyramidal tract consists of those fibers descending through the medullary pyramid to the spinal cord (cf 74, 17, 48) It is, com-

pared with other tracts that have been studied, a pathway of slow conduction velocity, at any rate in the cat The upper limit of the range of conduction velocities lies between 60 and 65 M per sec Velocities as low as 18 M per sec may be assigned to pyramidal tract fibers with reasonable certainty Assuming that the diameter velocity relations described for peripheral nerve fibers (21, 29) hold for fibers in the central nervous system, it is likely on the basis of histological evidence that fibers of still slower conduction velocity are represented, but their presence has not been demonstrated unequivocally by means of recorded action potentials

On stimulation of the medullary pyramid, after suitable precautions to prevent impulses in any tracts other than pyramidal from entering the spinal cord, it is found that interneurons at the base of the dorsal horn immediately adjacent to the pyramidal tract become active (cf the conclusion of Schäfer based on the evidence of degenerations (60)) The discharge of this nucleus by single shock stimulation of the pyramidal tract is insufficient to provide further spread of the activity, for no other activity is found on careful exploration with a microelectrode, nor are local reflexes in any way influenced. With repetitive stimulation activity is intensified and spread to other nuclei occurs When the system is fully active internuncial responses are confined to the dorsal horn and intermediate region The arrival of excitatory impulses at the intermediate region is accompanied by facilitation of three-neuron arc (i e, flexor) reflexes, without any parallel effect upon motoneurons observable. It is only after a nuclear delay of several milliseconds that discharges within the intermediate region occur, and facilitation of motoneurons parallels this discharge in the intermediate region. From this evidence it would appear that the primary action of the pyramidal fibers, active in isolation, is on the flexor reflex arcs.

*Corticospinal effects* Activity transmitted into the spinal cord directly through the pyramidal tract forms only a part of the total effect in the spinal cord of stimulating the pericruciate area of the (cat's) cerebral cortex. Tower (72) for instance, has described actions, both motor and inhibitory, on stimulating the pericruciate area (and other areas) after section of the medullary pyramids, and suggests the possibility that the reticulospinal tracts are involved in mediation of these extrapyramidal actions

One of the most striking sequelae of single shock stimulation of the motor cortex of the cat is the widespread activation of the reticular formation after a latency of approximately 4 msec (43) The exact pathway from cortex to reticular formation is not yet known but on the basis of time relations it is quite direct. The reticular activity is in turn projected by high velocity tract fibers to the ventral horn of the spinal cord It follows, then, that cortical control of the dorsal and ventral halves of the local spinal mechanism is dissociated at a prespinal level, impulses to the dorsal half being directly conducted by the pyramidal fibers themselves, those to the ventral half being relayed by reticular elements A simple calculation based on the known conduction rates and delays involved shows that cortically evoked activity through the reticular relays would reach the local spinal mechanism coincidentally with and earlier than the directly

conducted pyramidal impulses proper There is, then, the further possibility that cortical impulses and vestibulo-cerebellar impulses converge at the prespinal level, and then by the high velocity projection system 'set the stage' for the play of the pyramidal impulses arriving through the slower conducting pyramidal fibers

ALPHA FIBER SYSTEMS AND POSTURAL CONTROL Considerations such as that above serve to emphasize a correlation which seems of great potential significance (18, 19) The myotatic reflex pathway, and its associated projection through Flechsig's tract to the cerebellum, the vestibular and reticular systems and the dominant descending long spinal reflex pathways all have several dynamic properties in common They are characterized by the large size, high conduction velocity and relative uniformity of their constituent nerve fibers, and they are all primarily concerned with the transmission of postural activity In contrast, other reflex, afferent and motor systems, those mediating flexor reflexes, cutaneous sensitivity and voluntary motor activity are characterized by diversity of fiber size, with the largest fibers not overlapping to any great extent the range present in the postural mechanisms In general too the synaptic connections of these systems are more devious and complex than those of the postural mechanisms One is tempted to speculate on the functional significance of these facts for the integration of movement in the intact animal It seems quite plausible that the function of the high velocity systems set in parallel with other paths is in part to antecede those paths in temporal sequence of action and so to adjust posture to a state appropriate to the initiation of movement in readiness for the actual performance of that movement

INHIBITION Perhaps the thorniest of all problems connected with the study of nervous organization is that of clarifying the mechanisms of inhibition In general theories of inhibition postulate either a block of some part of the reflex pathway as the sequel to discharge or an inhibitory action of a direct nature for which the prototype is the long known vagal effect on the heart A theory (22) but recently expanded by Gesell (23) holds that there is a constant flow of current between dendrites and axon hillock, and that the action of impinging impulses (all presumably alike) is to increase or decrease the flow of current depending upon the anatomical relationship between the active synaptic knobs and the several parts of the responding neuron Depending upon the change in the current flow, either increasing or decreasing, the neuron is excited or inhibited Barron and Matthews (3) have suggested that polarization of axon junctions by adjacent activity may prove to be a true mechanism of central inhibition

Hughes and Gasser (28) and Gasser (18) point out that the subnormal period, discovered first in nerve by Graham (24), would account for the inhibition of the flexor reflex to the second of two successive single shock stimuli (16) In the same vein, Lorente de Nó (45) showed that a group of motoneurons, after antidromic activation, exhibit a prolonged period of subnormality, an event sufficient to account for the silent period following the transmission of a tendon-jerk reflex An important consideration for the occurrence of inhibition by the intervention of the subnormal period lies in the necessity for other than syn-

chronous convergence of interacting volleys, which event *ceteris paribus* would lead to summation

Following the demonstration by Hughes and Gasser (28) that the negative cord potential, signalling the activity of interneurons, and the reflex discharge to the second of two successive volleys are decreased in a parallel manner along a time course which follows that of the positive cord potential resulting from the first volley of the pair, McCouch, Hughes and Stewart (51) discovered that crossed inhibition of the flexor reflex may occur with little or no inhibition of the cord potential as recorded from the dorsal surface of the spinal cord, which fact leads them to the conclusion that inhibition in that case occurred at the motoneurons without conditioning by previous activity of the units involved Although this indeed may be the case, there is no evidence as yet by which to exclude other than direct inhibitory actions To interpret the experiments of McCouch, Hughes and Stewart it would seem important to know whether all of the interneurons which are required, in one way or another, for the transmission of the flexor reflex contribute sufficiently to the cord potential that their response may be assessed by inspection of the cord potential. It does seem likely that the experiments of McCouch, Hughes and Stewart preclude the possibility of crossed inhibition of the dorsal horn, whatever the mechanism, as the essential event in crossed inhibition of the flexor reflex, at least under the conditions in which the cord potential is not reduced with the reflex

Although it is valid to associate the negative intermediary potential and the flexor reflex, for the afferent components giving rise to the two events are similar (41), whether the association is one of casual relationships or of parallel relationships is not clearly defined by experiment Peripheral stimulation which gives rise to reflex flexion also gives rise to sensation, usually but not of necessity said to be painful There is seemingly no evidence upon which to state unequivocally whether the internuncial activity represented by the negative intermediary potential is primarily concerned with mediation of the flexor reflex or with mediation of sensation provoking impulses to ascending projection systems

Several examples of direct inhibition recently have been discovered, in each case the condition for the onset of inhibition being simultaneous convergence of the interacting volleys As first described, a single afferent volley in the sixth lumbar dorsal root was found to inhibit the two-neuron arc reflex evoked in the first sacral ventral root by a single volley in the first sacral dorsal root when the two dorsal root volleys were synchronized (35) Other examples of this form of central inhibition have since been disclosed (56, 38), and with one exception the inhibitory action has been unilateral and ipsilateral The exception was found in experiments on the third sacral segment in which a dorsal root volley on one side of the cord will inhibit the two-neuron-arc reflex evoked by a dorsal root volley on the other side of the cord when the two dorsal root volleys are synchronous (43) This crossed inhibition appears to be related to the bilateral nature of the effectors innervated by this segment

The afferent fibers yielding direct inhibition to the motoneurons are indistinguishable from those responsible for the two-neuron arc reflex discharge (39),

and belong, therefore, to the group of large low-threshold afferent fibers arising in muscle. Although a systematic survey has not yet been made, it appears from the known examples of direct inhibition that the low-threshold afferent fibers from a given muscle mediate two-neuron-arc excitation to the motoneurons of that muscle and direct inhibition to those of neighboring muscles. It is apparent, then, that the direct inhibitory action of muscle afferent fibers accounts for the silent period shown by Denny-Brown to occur in the gastrocnemius muscle as the reflex accompaniment of the tendon jerk in the quadriceps muscle (10).

The fact that inhibition of two-neuron-arc pathways occurs when stimulating and testing volleys are synchronous is in itself strong evidence for the existence of some form of direct inhibitory action, but still further evidence may be found in the fact that this inhibition occurs with threshold conditioning volleys (39), which are well below the size requisite to produce motoneuron discharges. If near-threshold conditioning dorsal root volleys are utilized the period of depression of a two-neuron-arc test reflex volley may last for 7 or 8 msec. (43) whereas, with larger conditioning volleys internuncial pools are powerfully activated and inhibition is truncated by the advent of facilitation. When a more physiological selection of motoneurons is made by utilizing an extensor muscle nerve rather than ventral roots for recording, an inhibitory period of more than 100 msec. may be realized (56) which suggests the participation of interneurons in the inhibitory conditionary activity.

In spite of the proven fact of direct inhibition, nothing that is yet known serves to indicate the mechanism entailed. There is no reason to suppose that distinct inhibitory fibers exist in the mammalian spinal cord, for the threshold and fiber spectrum of direct excitation and direct inhibition are identical (39). There is no evidence for or against the existence of specific inhibitory collaterals or synaptic knobs. In this connection, for example, it would be very valuable to know whether dorsal root fibers of the sixth lumbar segment have knobs distributed to motoneurons with axons passing into the first sacral ventral root. The answer to such questions as this, may provide the next step in reasoning on the nature of direct inhibition. Nothing is to be gained by supposing the existence of connections, or the site on the neurons of the supposed connections, and arguing therefrom. Until it is known whether or not direct inhibition involves synaptic connection and if so where the connections are on the neurons, little advance in the understanding of direct inhibition may be expected. It would seem to be within the province of histological investigation to provide the essential data.

The rôle of extrasynaptic influences in nervous organization. One cannot doubt but that fields of current about active neurons, and chemical diffusion gradients, have an influence upon the excitability of any neurons within the radius of effective action (22, 32, 57). Stimulation of nerve fibers by other nerve fibers under certain circumstances has been known since the experiments of Hering (27). Stimulation of nerve fibers by active muscle has been known even longer (49). Somewhat in analogy with the Matteucci experiment it has

been found that the intramuscular portions of some motor and afferent fibers are excited when a muscle is activated indirectly by synchronous nerve volleys (37). Following an antidromic volley to the motoneurons a small pseudo-reflex or recurrent volley is discharged to the periphery (55, 40). The recurrent volley appears only among the motoneurons which have carried the antidromic volley, it is initiated immediately following the termination of the absolutely refractory period of the motoneuron axon, and before the electrical signs of soma activity have subsided. There is some reason to believe that the more prolonged soma activity restimulates the axon, but a full and satisfactory account of the genesis of the recurrent volley is not yet available.

The dorsal root reflex (71) is clearly the result of restimulation, by some mechanism, of the afferent fibers. At some stage in the central course of the dorsal root reflex orthodox synaptic transmission is involved for the end product possesses the characteristics of synaptically transmitted activity. Similarly, in the example of active muscle stimulating certain intramuscular nerve fibers cited above, the activity resulting in those nerve fibers reflects fully the intervention of neuromuscular transmission as one step in the chain of events (37).

It is difficult to say which and how many of the effects just described are in essence artefacts produced by synchronous stimulation or other experimental procedures. As an instance it should be noted that a certain fraction of a muscle must be synchronously active before retrograde impulses appear in the motor fibers (37). One may hold, with complete justification, that the conditions for the genesis of retrograde impulses are never achieved in the course of natural motor performance. Again, in all the experiments for which the original observations of Hering form the prototype, damage resulting in a demarcation current appears to be the indispensable factor, in other similar experiments, chemical treatment (caffeine, strychnine or veratrine) or frank electrical polarization is the essential procedure.

So far the discussion has concerned adequate restimulation of nearby excitable elements by one means or another. With much less drastic experimental manipulation it has been found that more subtle threshold effects occur in some excitable elements when activity pervades neighboring elements. Katz and Schmitt (31), Blair and Erlanger (50), and Renshaw and Therman (58) have demonstrated threshold changes in fibers consequent upon the passage of impulses in contiguous fibers. Important as these observations are for the study of nerve excitability and conduction, the influences they demonstrate can hardly be regarded as determining the prime function of a nerve fiber, viz., the transmission of an impulse from one point in the body to another.

Within the neurophil extrasynaptic actions due to fields of current and chemical diffusion gradients are of potentially greater significance. A synchronous antidromic volley in certain motoneurons is known to affect the threshold to reflex activation of neighboring motoneurons (55). It would be important to determine finally whether this action is due to recurrent collaterals or to simple current flow about the active motoneurons. When the effect demonstrated by Renshaw is inhibitory, it is particularly effective among motoneurons supplying the parts

of a single muscle For example, stimulation of the nerve to one head of the gastrocnemius muscle inhibits reflex activity through the motoneurons of the other head It would be interesting to know if similar alterations in reflex excitability occur when neighboring motoneurons are activated in a completely random and asynchronous manner, as for instance during the course of a static stretch reflex

Renshaw's experiment seemingly accounts for the observation first made by Sherrington (65, 66) that simulation of one of several nerve branches to a muscle (after distal section) inhibits the innervated remainder of that muscle In Sherrington's experiment the motor axons as well as the afferent fibers unavoidably were stimulated, and the resulting impulses in the motor axons sweeping into the central parts of the motoneurons would have had the action described by Renshaw

It seems possible that fields of current or chemical diffusion gradients may only reach critical levels when a number of similarly orientated structures are simultaneously active With rotation of elements, diffuse activation and 'tonic' maintained discharge, the extrasynaptic influences might approach to the steady state in character and thus play no significant rôle in phasic activity On the other hand, it is possible that such influences would attain great significance for the long term 'state of excitability' of the nervous system One conceives, for instance, of the possibility that long term chemical readjustments lie behind the recovery from spinal shock

It is evident that a great deal of critical experiment and appraisal are needed before the true significance for central nervous system function of the extrasynaptic effects is understood

## CONCLUSIONS

One will readily appreciate from the preceding discussion the fact that most of the available information on functional organization relates to those systems consisting of large neurons equipped with axons of fairly high conduction velocity In the main this is due to the technical difficulties of exploring the finer fiber systems or regions populated with small cells This is particularly the case with respect to unmyelinated fiber systems, concerning which virtually nothing is known

Upon occasion functional study can determine in broad outline certain details of structure, sometimes it can solve problems to which a wealth of histological endeavor has provided no definitive answer, more often it can point out to the histologist fruitful lines of enquiry and define the problem to which an answer is sought It is a common occurrence for physiological progress to be frustrated by the paucity of histological data, but it is also true that the need for certain histological information can only be realized when the functional problem is defined This is now particularly the case in relation to the problem of direct inhibition There are, of course, regions where the synaptic relationships are well understood (cf 6) More often than not, unfortunately, the regions understood from a histological point of view at the present time are not amenable to functional study The converse is undoubtedly true

It would be difficult to overestimate the need for factual rather than hypothetical treatment of the problems of direct inhibition as a prerequisite for adequate and complete description of functional organization, and in particular for an understanding of the means by which the spinal mechanism is fractionated by various systems of reflex and higher control

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# OBESITY

## I ENERGY METABOLISM

L H NEWBURGH

*University of Michigan, Ann Arbor*

Obesity is that condition in which the body contains an abnormally large amount of adipose tissue The excessive fat may be evenly distributed or may be present to a much greater extent in some regions than in others When the fat is sharply localized to one or more discrete encapsulated masses, one speaks of "lipomatosis" in contradistinction to "obesity "

WEIGHT Since the accumulation of adipose tissue causes a corresponding increase in weight, much effort has been directed toward establishing a weight which is best in regard to longevity and mental and physical fitness

The Child Health Association (1) has compiled tables that give the average weight for height and sex of large numbers of healthy children from birth through the age of 18 years These tables are widely accepted as the most satisfactory standards

However, Fisk (2) has shown that the average weights of persons over 30 years of age are too great as judged by life expectancy and has found that the average weight at 30 is the most desirable weight for the remainder of the life Departures from these standards are sometimes condoned because of supposedly heavy bones, excessive muscular development or hereditary type, but according to Fisk, "Life insurance experience has shown that heavyweights, regardless of type and heredity, show an extra mortality "

Dublin and Lotka (3) have analyzed the influence of weight on the duration of life of 192,304 men aged 21 years or over when accepted for life insurance The deaths per hundred thousand, age being disregarded, are given in table 1 Dublin and Lotka (3) concluded that "the penalty of overweight is one-fourth to three-fourths excess in mortality "

These studies become still more informative when they are related to age, since excessive weight carries a much greater risk in persons beyond 45 years of age than earlier (table 2) How great the risk is for the important years from 45 to 50 appears dramatically in table 3 It is startling to learn that a mere 25 pounds (11 kgm ) of extra weight lessens one's life expectancy by 25 per cent Fish (2) found likewise that persons in full middle life who weigh 20 pounds (9 kgm ) more than the average instead of 10 pounds (4 5 kgm ) less are incurring an extra 25 per cent risk In another place he pointed out that "fifty pounds overweight at age forty-five imposes as much extra mortality as valvular heart disease "

ENERGY EXCHANGE Because of the prevalence of the belief that some patients gain weight even though they do not overeat and that others do not lose when they are underfed, there has been a continued search for some metabolic aberration in this disease

*Basal metabolism* Early students found that pound for pound obese persons produce less heat in the resting postabsorptive state than do normal controls Had they made the comparison on the basis of height, they would have found

TABLE 1

*The influence of weight on mortality deaths per hundred thousand men accepted for insurance*

| WEIGHT | DEATHS |
|---|---|
| Standard | 844 |
| Underweight total | 848 |
| Overweight, total | 1 111 |
| Underweight 5–14% | 833 |
| Underweight 15–34% | 913 |
| Overweight 5–14% | 1 027 |
| Overweight 15–24% | 1,215 |
| Overweight 25% or more | 1 472 |

TABLE 2

*Influence of weight on mortality as modified by age Deaths per hundred thousand*

| WEIGHT | AGE | |
|---|---|---|
| | Under 45 | Over 45 |
| Standard | 463 | 1 308 |
| Underweight total | 498 | 1 274 |
| Overweight total | 527 | 1 824 |

TABLE 3

*Influence of overweight on mortality in persons aged 45 to 50 years*

| POUNDS OVERWEIGHT | INCREASE IN DEATH RATE OVER AVERAGE |
|---|---|
| | *per cent* |
| 10 | 8 |
| 20 | 18 |
| 30 | 28 |
| 40 | 45 |
| 50 | 56 |
| 60 | 67 |
| 70 | 81 |
| 90 | 116 |

that the heat production of obese persons was greater than normal But Rubner, Lusk and others have demonstrated that the basal heat production of all mammals is proportional to the surface area of the body and that no such relation exists when either weight or height alone is used as the basis of comparison

Benedict, using the statistical method, has published tables by which one can predict the basal metabolic rate of normal persons on the basis of age, height and weight His values closely approximate those calculated by the surface area method There is, then, no doubt about the range of basal heat production of normal adults

Subsequently, Boothby and Sandiford (4) measured basal heat production in 94 obese patients and found that in 81 per cent of them the rates were within 10 per cent of the normal heat production per square meter In 3 instances the rate fell between −16 and −20 per cent, and another patient produced heat at a rate more than 16 per cent above normal Strouse, Wange and Dye (5) compared the basal metabolic rates of normal persons with those of subjects who were underweight and overweight Per square meter of body surface they found practically no differences Among 180 cases of extreme obesity Grafe (6) found only 3 in which there was a definite decrease in basal metabolic rate The occasional moderately low rate (from −15 to −25 per cent) exhibited by an obese person does not contribute to the understanding of obesity, since equally low rates are encountered as frequently among healthy persons who are not obese Strang and

TABLE 4

*Total basal heat production of five obese women compared with ideal values*

| | WEIGHT | SURFACE AREA | CALORIES/SQ M /HR | TOTAL NUMBER OF CALORIES/HR |
|---|---|---|---|---|
| | *lb* | *sq m* | | |
| Ideal | 129 | 1 59 | 36 5 | 58 |
| Observed | 238 | 2 06 | 35 5 | 73 |

Evans (7) made careful measurements of the basal metabolic rates of 5 obese women, and the average values are compared in table 4 with the predictions for normal women of the same average age The few obese persons whose basal metabolic rate is low enough to be definitely pathologic will be found to be suffering from some disease in which adiposity is a complication or an unrelated accompaniment and not the primary abnormality

These and many other studies lend overwhelming support to the statement that obesity is not caused by lessened expenditure of energy in the basal state In fact, an obese person produces more heat than a normal person of corresponding age, height and sex, for while both will produce the same number of calories per square meter of body surface per unit of time, the obese person has a larger surface, and therefore the total heat produced by the obese person in the basal state is greater actually than the total basal heat produced by the normal person

*Specific dynamic effect* If the heat production of a person who has been without food for twenty hours and who has been reclining quietly and comfortably for a half hour (basal state) is carefully measured and he is then fed, he will shortly produce more heat per unit of time than he did in the fasting state This response is not caused by digestion or absorption, since it is equally great after

the intravenous injection of dextrose or some of the amino acids that result from the digestion of protein If some metabolic fault prevented or greatly lessened this specific response to food, the person so afflicted would gain weight, provided he continued to partake of the same amount and the same kind of food and provided he did not increase his activity His appetite might, however, direct him to eat less, since he needed less, and in that case his weight would not increase Several students, without making any allowance for the second alternative, have attributed obesity to lessened specific dynamic effect of food

Many attempts have been made to show that the caloric response to food is controlled by the hypophysis Thus Plaut (8) stated, as a result of her studies, that a normal basal metabolic rate, coupled with a lowered specific dynamic response to protein, is characteristic of disease of the hypophysis Using these criteria, she expressed the belief that she (9) was able to show that in certain cases obesity was caused by hypofunction of the pituitary Kestner, Knipping and Liebesny subsequently published many determinations that seemed to confirm her work The technic employed by these investigators has been criticized by a number of workers Durr (10), for example, pointed out that a normal person exhibited no increased metabolic response to food in three hours and a response of only 18 per cent in five hours Lauter (11) in careful studies found that the specific dynamic response of normal subjects varies so widely that only its total absence is of diagnostic significance. Gaebler (12) working in Lusk's laboratory, found that dogs from which the hypophysis had been removed responded normally to a standard protein meal

Because of the disagreement Johnston (13) reinvestigated this question She restricted her studies to patients in whom destructive diseases of the pituitary were demonstrated, usually by operation or at autopsy She obtained large responses in all cases (18 to 28 per cent), and pointed out that it was not possible to secure uniform results in human beings. She found it impossible to obtain a consistent response to sucrose or aminoacetic acid in a normal subject maintained on a constant diet This again emphasized the caution with which small responses must be interpreted

Later work suggests that the liver is responsible for the increased heat liberated during the metabolism of protein Bollman and Magath (14) showed that deamination took place in the liver, and later, Wilhelmj and Mann (15) found that administration of amino acids to hepatectomized dogs did not increase the heat production Finally Dock (16), by excluding various portions of the bodies of rats from the circulation, found that at least 85 per cent of the specific dynamic heat was liberated in the abdominal viscera

The relation of the specific dynamic effect to the obese state has been studied so painstakingly and comprehensively by Strang and McClugage (17) that their results may be accepted with great confidence They emphasized that the base line, that is, the value of the basal metabolism, is the most important feature of the test Earlier workers had failed to train their subjects sufficiently Strang and McClugage have repeated basal determinations until uniform results were obtained In order to make a test acceptable they demanded that the basal

calories on the test day must vary less than 2 calories from the average of measurements within fourteen days On this basis they rejected ten of their twenty-five determinations They pointed out the fallacy of the usual way of expressing the specific effect as a percentage of the basal calories For example, a 10 calory increase attributable to food when the subject is producing 60 calories per hour in the basal state is an increase of 16 per cent, but the same specific increment with a basal heat production of 90 calories is an increase of only 11 per cent Since obese persons produce more heat in the basal state than do normal persons, the same absolute response to a test diet may appear to be low in obese persons when the percentile method of comparison is used It is likewise misleading to express the response to a test diet in terms of surface area Thus an increment of 10 calories due to food in a person whose surface area is 1 67 sq m gives an increase of 6 calories per square meter, whereas if the surface were 2 2 sq m , as is common in obese persons, the increase would be only 4 5 calories per square meter Hence, the same absolute increase in the heat results in an apparent depression for obese subjects These workers emphasized the irregularity of the response to food when viewed from hour to hour and were in agreement with Benedict and Carpenter (18) "that a true appreciation of the thermal effect of a meal is obtained by focusing the attention on the total increment" With these considerations in mind, Strang and McClugage studied 5 normal, 5 thin and 8 obese persons The total increments for the eight hours following the ingestion of the test meal will be found in table 5

TABLE 5

*Specific dynamic effect of food*

| TYPE OF SUBJECT | INDIVIDUAL VARIATION IN NUMBER OF CALORIES | AVERAGE NUMBER OF CALORIES |
|---|---|---|
| Normal | 43 to 61 | 51 |
| Obese | 44 to 74 | 58 |
| Thin | 59 to 78 | 67 |

Luxuskonsumption Grafe (19) has maintained that in addition to the specific dynamic response, which comes to an end in twelve hours or less, the heat production of the organism throughout the twenty-four hours is influenced by the quantity of food eaten Leaving the increases that accompany activity and the specific dynamic effect out of consideration, he expressed the belief that the intensity of the metabolism is stimulated by generous (excessive) feeding and depressed by meager supplies of food Such a mechanism would tend to maintain constancy of weight Obesity would develop when the mechanism failed to respond to overeating, and leanness would be the result of abnormally great responses

Grafe and Koch (20) reported a study on a patient whose normal weight was 62 6 kgm and whose height was 156 cm Because of stenosis of the pylorus accompanied by persistent vomiting his weight had fallen to 40 kgm In this

emaciated condition he produced 30 calories per square meter per hour in the resting fasting state The prediction for a man of his age and height and of normal weight is 39 calories per square meter Relief of the stenosis and subsequent feeding of 100 calories per kilogram brought his weight up to 60 kgm He then produced 40 7 calories per square meter, which was within 3 per cent of the prediction for a man of his age and height and of normal weight This study merely confirmed Schondroff's (21) and Zuntz' earlier work that starvation depressed the basal metabolism

The reader will recall that Grafe had said that the normal organism maintains a constant weight in spite of overfeeding because the extra food stimulates the total metabolism to such an extent that the excess is oxidized, but he did not publish any data that dealt with total heat production throughout the twenty four hours In order to test Grafe's postulate Wiley and Newburgh (23) studied the responses of a thin person by means of a method (24) recently developed for determining the total heat production for any desired length of time

The subject was 28 years old, 6 feet (183 cm ) tall and weighed 45 pounds (20 kgm ) less than normal For the first eighteen days he was fed a constant diet that yielded 2,922 calories daily During this same period the twenty four hourly heat production averaged 2,947 calories His weight at the beginning was 57 562 grams, and at the end it was 57,548 grams He was then fed a diet that yielded 4,755 calories daily This was an increase of 1,833 calories During the fifteen days the latter diet was taken the subject gained 4,410 grams (nearly 10 lbs ) Heat production per twenty four hours in the second period averaged 3,082 calories Accordingly, he produced 135 more calories when he was ingesting about 5,000 calories than when he took about 3,000 calories, an increase of 4 5 per cent in total metabolism The increase in the surface area that accompanied the increase in weight accounted for 68 extra calories and the additional carbohydrate and fat could be expected to increase the specific dynamic effect of the second diet by 85 calories, according to a calculation by Lusk (25) Therefore the predicted increase in heat production during the second period without recourse to Luxuskonsumption is 153 calories, whereas the actual increase was only 135 calories When the subject was partaking of the smaller diet, the fasting resting heat production was at the rate of 35.5 calories per square meter per hour At the end of the period of overfeeding he produced 36 9 calories under the same conditions The difference of 1 4 calories is not significant These workers obtained no evidence that either the basal or the total metabolism was stimulated by superalimentation

*Total metabolism* It has repeatedly been observed that some obese patients who are receiving only minimal quantities of food fail to lose weight. Von Noorden (26) reported such experiences many years ago More recently Grafe (6) has published several examples, one of which is reproduced here in part

The patient, aged 38 was 5 feet 4 inches (163 cm ) tall and weighed 378 pounds (171 kgm ) She became stout at 10 years of age When she was 20 years old, she weighed 176 pounds (80 kgm ) She married at this time Subsequently, she gave birth to 4 babies and gained weight after each delivery When first seen by Grafe she exhibited enormous adiposity of

the trunk, legs and upper arms During the first month of treatment she lost 30 pounds (14 kgm ) Then without change in treatment the loss of weight gradually diminished, and finally, for two weeks there was no loss at all The weight remained stationary at 341 pounds (155 kgm ) During these two weeks her diet contained 647 calories, and the basal metabolism was 2,000 calories Grafe calculated that the daily caloric deficit was about 2,000 In addition to the restricted diet her fluid intake was limited to about 500 cc daily Under these circumstances the body should have lost 8 5 pounds (4 kgm ) of adipose tissue during these two weeks Even though the patient received four injections of mersalyl solution and 3 to 8 mgm of thyroxin daily, the volume of urine was small, averaging 500 cc daily She continued the treatment at home in a little milder form and lost 13 pounds (6 kgm ) in the next few weeks

This capacity to resist loss of weight in spite of the most rigorous treatment has given rise to much speculation and confusion Many physicians have considered it useless or even cruel to continue to underfeed patients of this type, since they seem to be doomed to hopeless adiposity The writers have seen many examples of this phenomenon Such paradoxic conduct might arise from some obscure metabolic abnormality that permits conservation in the utilization of energy In that case, the total heat production would be strikingly less than normal But no measurement of the twenty-four hour expenditure of energy had been published prior to the studies of Newburgh and his associates Such patients might have been studied in a respiration chamber for one or possibly a few consecutive days But the restricted activity and short intervals of time imposed by the chamber made that method of doubtful value for this special purpose They accordingly employed the method (23) previously cited by means of which they could measure the total loss of heat for many consecutive days while the usual activity continued Included were subjects with various types of obesity described in the literature, that is 1, a person physically normal except for obesity who frankly admitted years of gluttony, 2, a feeble-minded girl with a low basal metabolic rate, 3, a girl with disease of the pituitary body and a basal metabolic rate 30 per cent below normal, 4, a middle-aged woman whose weight had reached 295 pounds (134 kgm ) after an operation on the hypophysis eight years earlier, 5, a young woman suffering from so-called "Dercum's disease", 6, a middle-aged woman 5 feet 2 inches (157 cm ) tall whose weight had reached 420 pounds (191 kgm ) (menopausal obesity) In no case did they find anything unusual about the total metabolism These patients certainly did not exhibit any capacity to live at a lesser expenditure of calories than normal persons In fact, just the opposite was true for the patients who were active The total expenditure was large and indicated that they produced considerably more heat than persons of the same height, age and sex whose weight was normal The data are entirely in accord with those of Lauter (11) who found that an obese subject requires more energy to perform a given piece of work than does a normal control

WATER BALANCE In spite of the large expenditure of energy, periods lasting for a number of days during which the patients failed to lose weight, even though the calories of the diet were far less than the dissipation of heat, were frequently encountered A good example of this phenomenon is illustrated by figure 1

The patient was a young woman who weighed 275 pounds (125 kgm) when she entered the hospital. Her basal metabolism, which was normal, amounted to 2,100 calories per twenty four hours. Throughout the study she remained in bed. In spite of the inactivity the total twenty four hourly heat production averaged 2800 calories. She received a diet that yielded 1600 calories daily. She was accordingly compelled to oxidize enough body tissue to produce 1200 calories daily. After a preliminary week on the diet, the analyses showed that about 150 grams of body tissue were destroyed daily. This weight times the number of days subtracted from the weight of the subject at the beginning of the

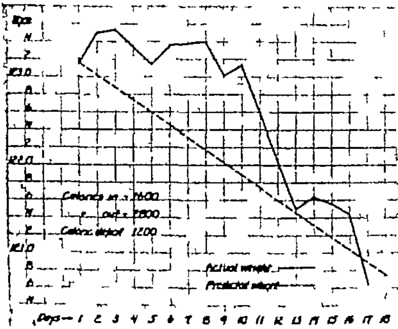

Fig 1

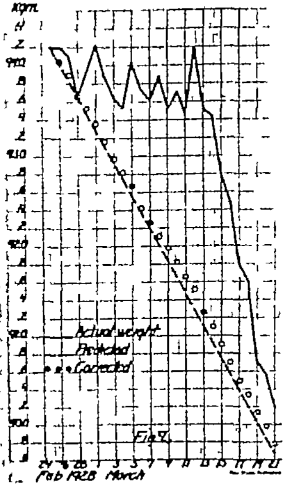


Fig 2

experiment indicates what she would have weighed on any day if nothing else had intervened to disturb these simple relations. This hypothetic weight is represented in the diagram by the broken line. The solid line is the actual day to day weight of the patient. In spite of the underfeeding, the patient weighed approximately as much on the morning of the tenth day as she did on the first. In fact, she actually gained the first and the second day. This capacity to resist loss of weight, even though body tissues are certainly being oxidized and the end products excreted, may last two weeks or longer. But whatever the duration, it always gives way to a subsequent rapid loss of weight equal to the weight of the

tissue destroyed over the entire period of caloric restriction In the diagram it will be seen that this patient lost as much weight in three days (tenth, eleventh and twelfth) as she was expected to lose in twelve days, due to destruction of body tissue

It seemed to us, a priori, that this phenomenon might be caused by a retention of water subsequently followed by excretion of the excess Accordingly, we undertook a study of the water balance (27)

In order to control our work we first studied the water relations in a normal subject while he was being underfed For the sake of uniformity he remained in bed except when he took a few steps to the scale or the commode To minimize error his diet was restricted to milk, sugar and water He received 63 grams of protein, 26 grams of fat and 148 grams of carbohydrate, yielding 1,078 calories He produced 1,688 calories daily, derived from 69 grams of protein, 91 grams of fat and 148 grams of carbohydrate (averages for each twenty-four hours) Consequently, he destroyed 6 grams of body protein and 65 grams of body fat each twenty-four hours The combined weight of these two substances in their

TABLE 6

*Concealment of destruction of tissue by retention of water*

| DAY OF EXPERIMENT | CHANGE IN WEIGHT OF SUBJECT | WEIGHT OF BODY TISSUE DESTROYED | WATER BALANCE |
|---|---|---|---|
| | *grams* | *grams* | *grams* |
| 1 | +285 | 95 | +369 |
| 2 | −225 | 95 | −105 |
| 3 | +65 | 95 | +149 |
| 4 | −125 | 95 | −41 |
| 5 | +115 | 95 | +198 |
| Totals | +115 | 475 | +570 |

hydrated living state is 95 grams It was soon noticed that this man, even though normal, failed to lose weight smoothly Like obese persons, for several consecutive days he would lose no weight or he might even gain weight Then his weight would suddenly decline at a rapid rate far in excess of 95 grams daily Table 6 shows the water exchange for five consecutive days when the subject weighed 115 grams more at the end than he did at the beginning, even though he had destroyed 475 grams of body tissue It is quite clear that the failure of the body to keep pace with the loss of tissue is attributable to the retention of water

Since these events took place in a normal person, they are deprived of all specificity for obese subjects The excessive retention of water is seen to be merely a by-product of undernutrition It in no way indicates that the metabolism of obese persons is abnormal

An analogous response by an obese girl is portrayed in figure 2 It will be noted that she weighed as much on the fifteenth day as she did at the start Then a marked diuresis set in and continued for the next nine days, by which time she had lost an amount of weight that approximately equaled the weight

of the body tissue destroyed during the twenty four days The solid line in figure 2 indicates the actual weight each morning The broken line states what she would have weighed in response to the destruction of body tissue, if the water balance had not been disturbed The position of the circles was calculated by subtracting the weight of the extra water in her body from the actual weight They show that her ability to resist loss of weight for more than two weeks was caused by progressive addition of water, which finally amounted to more than 6 pounds (3 kgm )

INCREASED ABSORPTION OF FOOD After it had been generally accepted that obese persons produce more heat than normal controls, the possibility still remained that if the digestion and absorption of food by obese persons was sufficiently more efficient than that by normal people, the former could succeed in gaining weight without overeating Nenenschwander Lemmer (28) has investigated this possibility He compared the combustible materials in the feces with those in the diet The utilization of the dietary calories, nitrogen and fat by the obese persons was 87, 84 and 83 per cent respectively For the controls,

TABLE 7

*Blood levels of fats*

| CONDITION OF SUBJECTS | USUAL DIET | | DIET PLANNED TO INSURE UNDERNUTRITION | |
|---|---|---|---|---|
| | Range | Average | Range | Average |
| | *mgm per 100 cc* | *mgm per 100 cc.* | *mgm per 100 cc* | *mgm per 100 cc.* |
| Obese | 544 to 1 117 | 890 | 446 to 802 | 630 |
| Normal | 371 to 1 005 | 591 | 414 to 997 | 607 |

the corresponding values were 88, 86 and 80 per cent Obesity was not caused by an economy of food

"LIPOPHILIA " Von Bergmann suggested that adiposity is caused by a hereditary constitutional trait of the adipose cells that enables them to accumulate excessive amounts of fat Hetenyi (29) carried this idea one step further by postulating that fat once deposited in the depots of an obese person is prevented from leaving them and so cannot be used as fuel Increased appetite is the natural response To support this contention, he compared the levels of the blood fats in obese persons with those of normal persons when the usual food was taken and again after eight days of restricted diet The data appear in table 7

Since obese persons have more fat in the blood when food is unrestricted, they must be either storing less or mobilizing more of it than normal persons The lowering of the blood fat level in obese persons by underfeeding is accepted by Hetenyi as evidence that they have difficulty in releasing fat from the depots in response to the increased call for energy, but it might just as well mean that fat which is being mobilized at a normal rate is being oxidized more rapidly Later studies dealt with blood fat curves after feeding 200 cc of cream The average increase in the controls amounted to 84 per cent of the fasting values as compared

with an increase of only 17 per cent in the obese subjects The indivic sponses, however, varied from zero to 157 per cent, and such a wide va throws considerable doubt on the value of the averages The lower level obese subjects are offered by Hetenyi as evidence of increased avidity fo the depots, but since the greater metabolism of obese persons demands mo why do not the lower levels of fat indicate accelerated oxidation of it?

If it were true that the adipose tissue cells of obese persons resist mobi of fat in undernutrition, then such persons would not lose weight, or if th most of the loss would represent the destruction of body protein But lowing studies show that obese persons are less likely to go into negative n balance when underfed than are normal subjects Jansen (30) placed ical students whose average weight was 62 kgm on a diet yielding 1,600 and 61 grams of protein daily for several weeks Their average loss of n was 2 grams daily Benedict's (31) 12 normal subjects receiving 1,534 and 51 grams of protein lost 65 grams of nitrogen from the body in three On the other hand, Keeton and Dickson (32) showed that most obese pers diet yielding 1,375 calories and 90 grams of protein maintained nitrogen and Strang, McClugage and Evans (33) found that obese persons receiv 440 calories and about 1 gram of protein per kilogram of ideal body weigh lose body protein

With these things in mind our colleague, Malcolm Block, has reinvestig response to underfeeding After determining the total blood lipids duri liminary period when the diet was unrestricted, he placed 3 normal young and 3 obese young women on a series of diets The first diets were arra yield approximately 80 per cent of the energy of the basal heat produc twenty-four hours After seven days, blood lipids were determined ag the diets were reduced to about 60 per cent of the basal heat Sev later, samples of blood were again withdrawn and the calories of the di reduced to 40 per cent of the twenty-four hourly basal heat production end of this week, samples of blood were obtained and the obese patie all placed on a diet yielding 450 calories The blood fat readings are rec table 8 An examination of these values indicates that they rose and both in the obese patients and in the controls, and it is not possible to de significant difference in the responses of the two groups to underfeed addition, Block calculated the dissipation of heat from the insensibl weight (24) and estimated the caloric deficit by comparing the energy of with the expenditure of energy The differences were then converted t of body tissue destroyed, on the assumption that it consisted entirel The results for the 3 obese patients are found in table 9, which also rec daily intake of nitrogen and the urinary nitrogen It will be seen tha the patients lost almost exactly the amount of weight that she was exp lose if the body tissue destroyed to make up the caloric deficit was solely tissue Furthermore, the comparison between the dietary and the urina gen shows that the patients lost only negligible amounts of body protein

All of these later studies indicate that obese persons release fat from t

TABLE 8

*Variation in total blood lipids in three normal and three obese young women on various diets*

| DIET | BLOOD LEVELS OF FATS | |
|---|---|---|
| | Normal controls | Obese subjects |
| | mgm /100 cc | mgm /100 cc. |
| nrestricted | 490<br>523<br>610 | 700<br>540<br>500 |
| per cent of basal calories | 706<br>750<br>710 | 808<br>575<br>700 |
| per cent of basal calories | 846<br>843<br>806 | 808<br>700<br>850 |
| per cent of basal calories | 703<br>605<br>542 | 910<br>850<br>610 |
| per cent of basal calories | | 710<br>910<br>608 |

TABLE 9

*oss of weight and daily dietary intake and urinary output of nitrogen in three obese subjects*

| MBER OF SUBJECT | DAILY LOSS OF WEIGHT | | DAILY NITROGEN | |
|---|---|---|---|---|
| | Predicted | Actual | Intake in diet | Output in urine |
| | grams | grams | grams | grams |
| 13 | 254 6 | 255 4 | 13 81 | 14 34 |
| 14 | 250 7 | 249 2 | 13 81 | 13 35 |
| 15 | 222 8 | 222 8 | 13 66 | 13 75 |

TABLE 10

*Respiratory quotients after a test meal*

| | HOURS AFTER MEALS | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 6 | 8 |
| rmal subjects | 838 | 810 | 804 | 823 | 812 | 819 |
| eso subjects | 746 | 772 | 752 | 740 | 761 | 795 |

a source of energy as readily as normal persons do In fact, it seems likely at the obese persons do this more readily, since they usually remain in nitrogen lance when they are underfed, whereas persons of normal weight do not

The fasting respiratory quotients in obese persons are lower than they are in normal controls According to the usual interpretation, this means that the former persons are oxidizing more fat Quotients published by Strang (34) are enlightening The fasting values obtained from 7 obese patients ranged from 0 698 to 0 830 and averaged 0 757 The controls under similar conditions ranged from 0 770 to 0 848 and averaged 0 783 These same persons were studied again after a test meal consisting of 40 grams of protein, 26 grams of fat and 52 grams of carbohydrate Quotients were obtained repeatedly after the meal for eight hours and are presented in table 10

Since both the quotients obtained during fasting and those obtained after the ingestion of food are lower in obese subjects, we have before us classic evidence that such persons are metabolizing more fat than the normal controls They cannot at the same time be storing more fat or withholding more of it in the depots

## SUMMARY

These many painstaking investigations of the metabolism of obese persons have failed to disclose any abnormal process that accounts for the accumulation of the fat On the contrary, they have demonstrted that obese persons produce more heat in the basal state, that they expend more energy to perform a measured amount of work and that their total heat production is greater than that of normal persons of similar age, height and sex under the same circumstances Since they are unable to absorb more energy from their food, they must eat more than normal people simply to avoid loss of weight

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## II ETIOLOGICAL ASPECTS

J W CONN

*University of Michigan Ann Arbor*

The foregoing presentation of the various aspects of energy exchange leave no retreat from the commitment that all obesity, in the final analysis, is the result of an overall inflow of energy which has exceeded the overall outflow, with a resultant retention in the body of the excess energy as fat The question of the fundamental etiology of obesity, therefore, must concern itself not with whether but with why there occurs a positive energy balance Are there various mechanisms which may be responsible for the initiation and continuation of positive energy balance? An answer supported by unquestioned experimental evidence as regards the human being cannot now be given, but the various possibilities that exist will be set forth in the succeeding paragraphs The fact remains, in any case that, regardless of the mechanism involved in its production, all obesity (clinical as well as experimental) responds with a predictable loss of body fat to measures which reduce energy intake below energy expenditure

Many of the hypothetical mechanisms alleged to result in the production of obesity are nebulous and devoid of experimental confirmation An attempt will be made to review those concepts which in the author's opinion deserve consideration Since it is taken for granted that the body fat does not arise spontaneously, and that every gram of body fat had an isocaloric precursor which entered the body from without, we shall omit those concepts which insist that the human body has learned to outwit the fundamental laws of thermodynamics

Finally, with regard to thermodynamics, a brief definition of terminology is necessary in order to provide proper insight When one speaks solely in terms of energy equilibria, he is indicating the *end result* of a balance sheet in which

the only considerations involved are total caloric intake, total caloric output and change in body weight (taking into consideration water exchange for the period) From this point of view all obesity is necessarily of one kind, that is, a positive energy balance phenomenon This is indisputable But in the sense that changes in caloric intake, caloric expenditure or both might be influenced, directly or indirectly, by psychic, hereditary, endocrine or metabolic aberrations and by functional or organic changes in the central nervous system, various types of obesity are possible It is with these considerations in mind that we proceed to examine the evidence

Hypothalamic obesity Rather intriguing and recently more clearly defined is the experimental production of obesity in normal animals in which bilateral lesions of the hypothalamus have been produced Beginning with Erdheim (1) in 1904 many clinical reports have appeared suggesting that destructive lesions involving the floor of the third ventricle and either including or sparing the hypophysis have resulted in marked obesity Working with dogs Camus and Roussy (2, 3) followed by Baily and Bremer (4) and later by Keller and Noble (5) noted the development of obesity following experimental injury to the posterior hypothalamus The hypophysis was carefully avoided and it was concluded that the obesity produced was the result of the hypothalamic lesion The recent work in dogs by Heinbecker and White (6) appears to delineate with greater specificity the hypothalamic area involved They found that lesions of the posterior hypothalamus, caudal to the paraventricular nuclei, which interrupt fibers whose cell bodies originate in the caudal portions of the paraventricular nuclei (resulting in retrograde degeneration of these structures) result in obesity, that similar lesions not producing the retrograde degeneration in this region did not produce obesity They noted, too, that the coexisting loss of the cells of the supraoptic nuclei (producing diabetes insipidus) resulted in the development of a greater degree of obesity (75 per cent to 110 per cent increase in weight in 6 months as compared with 50 per cent for the paraventricular nuclear lesion alone) They conclude that the specific obesity lesion in the dog is a diminution in the number of cells in the caudal portion of the paraventricular nuclei, that in the presence of such a lesion a simultaneous decrease in posterior pituitary secretion intensifies the resulting obesity

In a recent report, Brooks, Lambert and Bard (7) indicate the development of obesity in six of twelve monkeys (Macaca mulatta) following experimentally produced hypothalamic lesions Mature monkeys more than doubled their weight in 8 to 10 months, after which the weights remained relatively constant The presence or absence of an associated diabetes insipidus or hypogonadism did not influence the development of obesity The total caloric intake of the obese monkeys was much greater than that of their non-obese controls

Much more extensive observations on this type of obesity have been made in the rat Smith (8, 9) had shown in 1927 that extreme obesity could be produced in rats by hypothalamic lesions placed so as to avoid the hypophysis By means of the Horsley-Clark stereotactic instrument fairly standard hypothalamic lesions are now produced and obesity develops with great regularity

From a series of experiments by Hetherington and Ranson (10, 11, 12) the following conclusions can be drawn 1, destruction of the hypophysis of the rat does not result in the production of obesity, 2, large, symmetrically placed lesions of the hypothalamus produce obesity in virtually 100 per cent of rats so treated, 3, obesity produced by bilateral hypothalamic lesions are not dependent upon intact nervous pathways between the hypothalamus and the hyphophysis nor upon the presence or absence of the hyphophysis They believe as do Heinbecker and White (6) (the latter used dogs) that the obesity is dependent upon the interruption of descending fibers which leave the ventromedial hypothathalmic nuclei and normally descend toward the brain stem

A few studies of the metabolism of animals made to become obese by experimentally produced hypothalamic lesions have been made As mentioned above, Brooks et al (7) noted a greatly increased caloric intake in their operated monkeys When deprived of food the obese monkeys lost weight slightly less rapidly than did the non-obese monkeys Hetherington and Ranson (11) studied the spontaneous activity and food intake of rats with hypothalamic lesions Two things were clear 1 All of the operated animals were much less active than their controls 2 Most of the animals ate a great deal more than their controls Because some of the animals became obese without eating more than their controls the authors seemed unduly troubled The lowered activity could easily account for the apparent discrepancy This decreased activity probably explains why Brooks' obese monkeys (7) failed to lose weight as rapidly as their controls when both were deprived of food

The excellent work of Long and his associates (13–18) upon metabolism in hypothalamic obesity may be summarized as follows 1, shortly after the hypothalamic lesions were placed, the animals began to consume 2 to 3 times the amount of food eaten by their littermate controls and became extremely obese, 2, when paired fed with their controls, only 1 of 10 operated animals outgained its control, 3, studies of basal and total oxygen consumption and of basal and postprandial R Q 's in paired fed operated (non obese) and obese operated rats showed no deviations from the normal, which were not fully accountable on the basis of increased body weight and the eating habits of the operated animals, 4, "the development of obesity is apparently a consequence of increased appetite, and is not associated with any fundamental disturbance in metabolism "

Thus, it appears that in at least three mammalian species (rat, dog and monkey) experimentally produced destructive lesions of the hypothalamus which sever descending nervous pathways from the paraventricular nuclei lead to the rapid development of obesity Clinical examples of this phenomenon, although not as well studied or as easily controlled, may be found in the literature An adequate explanation for the resulting obesity is found in the tremendously increased food intake and to a lesser degree in the concomitant decrease in spontaneous activity exhibited by experimental animals That there has occurred no significant change in fundamental metabolic processes may be adduced from the facts 1, that operated animals paired fed with their

controls behave, with regard to gain in weight, like the controls, 2, that operated animals deprived of food lose weight at the expected rate, any slight deviation probably being accountable on the basis of decreased activity of the operated group, and 3, that oxygen consumption and R Q studies on such animals reveal no deviations from the normal

The conclusion seems irresistable that dysfunction of nervous elements which originate in or are mediated through certain nuclei of the hypothalamus is capable of producing a tremendous increase in the desire for food By what means normal function of this system controls the hunger mechanism or how its destruction releases the inhibitory control remain for the present unknown That an organic mechanism exists is evident That this mechanism plays a significant rôle in the etiology of human obesity is not evident That it cannot be disregarded, however, as a possible factor in human obesity is equally clear

ENDOCRINE OBESITY 1 *Effect of endocrine function upon energy balance* It has gradually become apparent that one cannot think in terms of hyperfunction or hypofunction of any one of the endocrine glands without, at the same time, considering the effect of the specific dysfunction upon the remaining glands of internal secretion In this sense most endocrine disturbances are polyglandular, even though replacement therapy for a primary hypofunction may result in return of the whole system to normal It seems reasonable to state that until a specific metabolic aberration which can lead to obesity is demonstrably related to a given endocrine dysfunction it is premature to attach significance, from an etiological point of view, to such time-worn expressions as pituitary obesity, gonadal obesity, etc It is true that certain non-specific metabolic changes such as hypoglycemia or reduced basal heat production may be instrumental in stimulating increased intake of energy or in decreasing energy expenditure But such metabolic changes may result from a variety of types of endocrine imbalance When obesity does occur in association with some such metabolic change it would be more realistic to term it "low energy output obesity" in the hypometabolism case and "high energy intake obesity" in the hypoglycemia case, both cases evidencing a positive energy balance To say "hypothyroid obesity" implies that hypothyroidism per se produces obesity As a matter of fact, most patients suffering from the severest form of hypothyroidism, myxedema, do not become obese (19) They automatically adjust caloric intake downward in accordance with the decreased need for energy

One can analyze, in a similar way, the example of hypoglycemia The following provides an interesting comparison of two such patients upon whom we (20) have recently concluded prolonged metabolic studies

1 Each had been suffering for several years from daily attacks of severe and prolonged hypoglycemia due to pancreatic islet cell tumors Blood sugar levels during attacks varied in each between 10 and 40 mgm per cent and the fasting blood sugar value was almost always below 50 mgm per cent

2 One had maintained a normal weight throughout his illness, hunger not having been a prominent symptom of his hypoglycemia The other had gained 50 pounds since the onset

of her illness 20 pounds of which had been added in the past year She had found that ingestion of food delayed or alleviated attacks

3 Each successful surgical removal of the insuloma restored carbohydrate metabolism to normal Each however *maintained* his pre-operative weight i e the obese woman remained obese despite removal of the initiating cause of her positive energy balance

4 Because of her obesity a reduction diet was prescribed She lost 30 pounds but soon her desire for more food overcame her desire to lose more weight and she gradually gained back what she had previously lost

It is evident that the metabolic change occasioned by the abnormal endocrine function resulted in obesity only when it caused increased ingestion of food, that removal of the metabolic aberration did not result in loss of weight, and that the food habits, acquired during the hypoglycemic period, persisted as a cause of obesity after removal of the initiating influence

One is obliged, therefore, to interpret the endocrine element in the production of obesity in terms of its effect upon total exchange of energy, remembering that the same dysfunction may not affect the energy balance of two individuals in the same way The endocrine dyscrasia is not the thing that produces accumulation of adipose tissue

2 *Effect of endocrine function upon distribution of body fat* Superficial observation alone is sufficient to convince one that the distribution of body fat is significantly influenced by the endocrine status of the individual. Patients previously normal suddenly begin to redistribute body fat in rather constant patterns soon after the onset of certain endocrine disorders A classical example is found in the case of Cushing s syndrome in which obesity of the face and neck become prominent along with loss of subcutaneous fat from the extremities When positive energy balance exists and there occurs an actual increase in total body weight, the added fat is distributed, for the most part, in the upper half of the body The total body weight, on the other hand, may be normal or subnormal and yet the obesity of the face and neck is sufficient to suggest that the patient is greatly overweight (see figs 1 and 2)

Similarly all who have observed the remarkable change that occurs in a sexually mature female who develops a virilizing tumor of an ovary, must agree that whatever may be the hereditary gene pattern for specific bodily form the influence of normal or abnormal endocrine function is an important determinant One is all the more convinced when normal female distribution of fat once again returns upon removal of the ovarian neoplasm

No one doubts that two perfectly normal individuals, one male and one female will become obese if fed 1000 calories in excess of their daily requirements for a sufficiently long period If this is done at age 6, the distribution of deposited fat will be general and almost identical If carried out after sexual maturity on the other hand the localities of fat distribution will be significantly different The influence upon regional distribution of fat has been exerted by a difference in endocrine function [1]

[1] *Regional lipophilia* Much has been written but little is known concerning the local mechanisms which allow one region of the body to become a fat depot while preventing such accumulation of fat in another It is interesting to remark in passing however that in

Thus, with regard to the endocrine influence upon obesity, two factors must be sharply distinguished (1) the influence of the disturbance in increasing food intake and/or decreasing energy expenditure, (2) its influence in determining regional distribution of fat The former is much less important than the latter That is, individuals with a given endocrine lesion may or may not be obese But obese individuals possessing certain endocrine abnormalities are likely to exhibit a fairly characteristic distribution of body fat The examining physician must, therefore, pay close attention to the distribution of fat in any obese individual It will occasionally lead him to the *additional diagnosis* of a specific endocrine disease

Fig 1

Fig 2

*Hypophysis* 1 *Pituitary obesity* This once popular clinical diagnosis is fortunately gradually disappearing It was used to attach an etiological tag to the previously described syndrome of adiposo-genital dystrophy This syndrome of obesity and hypogonadism sometimes seen in patients who have destructive lesions in the region of the stalk of the pituitary gland is reproducible in animals by means of experimental lesions at the base of the brain close to pituitary infundibulum (see hypothalamic obesity) That production of this complex is independent of the presence or absence of the pituitary gland has been demonstrated (8, 12) It appears from the evidence at hand that clean removal of the pituitary (experimentally) or destructive lesions of the gland (clinically), which do not injure important adjacent hypothalamic nuclei,

humans in whom an abnormal distribution of fat can be etiologically related to a known endocrine dysfunction the steroid hormones, adrenal cortex or gonads, appear almost invariably to be involved

produce progressive emaciation and cachexia (8, 21, 22) and that dysfunction of hypothalamic centers is required for the development of adiposo-genital dystrophy The mechanism by which genital dystrophy results from hypothalamic injury when the pituitary gland remains intact needs further study

It is to be recalled that the thyroid and adrenal cortex which also depend for their integrity upon intact pituitary function, are not anatomically disturbed by the experimental lesion which produces adiposo-genital dystrophy (8) Hypophysectomy, as is well known, results in hypoplasia of all three (thyroid, adrenal cortex and gonads)

Rony (23) and Schwartz et al (24) have reported that the administration of gonadotropic substances to patients with adiposo-genital dystrophy has no effect upon the adiposity even though it does induce genital development One is forced to conclude from the available information that in true adiposo-genital dystrophy the increased desire for food (and consequently the obesity) is the direct result of hypothalamic dysfunction, that the accompanying genital dystrophy may involve a specific effect of the hypothalamic lesion upon the gonadotropic function of the anterior pituitary gland, or that the obesity per se has affected genital development In the latter connection, the frequent occurrence of delayed puberty in obese youngsters needs further emphasis (see under Gonads)

2 *Cushing's syndrome* This special situation has been discussed in some detail above From a metabolic point of view it would appear that the interesting clinical picture is produced by prolonged increased secretory activity of the adrenal cortex (25) That in some cases the lesion is primary in the adrenal cortex is clear (26) That an indistinguishable syndrome occurs in association with basophilic adenomas of the pituitary gland has also been shown (27) The connecting link is found in the fact that a large percentage of the latter group of cases have developed hypertrophy of the adrenal cortices (28) Since Cushing's syndrome, even when secondary to "pituitary basophilism", appears to exist by virtue of hyperadrenocorticism, there is no reason to incriminate the pituitary gland for any one of the many characteristic manifestations of the disease

*Adrenal cortex* A characteristic type of distribution of body fat occurs with great regularity in patients with Cushing's syndrome As indicated above the syndrome has been interpreted as being due to hyperadrenocorticism (25) The peculiar distribution of fat has attracted comment of virtually all who have studied these patients The characteristic appearance has led to the beautifully descriptive term of humpty-dumpty disease" We (29) have recently conducted a prolonged metabolic investigation of such a patient and present his appearance as a typical example (figs 1 and 2) The striking accumulation of fat about the face, neck, shoulders and upper chest has been noted almost invariably[2] Contrary to expectations from outward appearances, however,

[2] An opportunity was afforded to demonstrate that this tissue is actually fat An abscess developed in the subcutaneous tissue beneath the chin At the time of incision for drainage, a thick mass of adipose tissue was found to extend as far as the probing finger could reach

there is very little adipose tissue under the abdominal skin The protruding abdomen appears to be due to loss of tone of the abdominal musculature Shortening of the thoracic spine due to collapse of vertebral bodies (where this has occurred) intensifies the appearance of obesity of the trunk Thus, the total amount of subcutaneous adipose tissue and, in fact, the total weight of the patient, may be below average But, overweight or not, the amount of fat about the face, neck and shoulders is excessive [1] Loss of body weight by caloric restriction proceeds normally (30) The basal metabolic rate, though frequently low, is variable

A metabolic aberration in the form of excessive glyconeogenesis from protein with a resultant unavailability of amino acids for replacement of essential tissue needs (all due to hypersecretion of adrenal steroids) has been hypothesized by Albright (25) to account for many of the physical abnormalities observed in such patients He refers to Woodyatt's finding (25) of negative nitrogen balance despite high protein feeding in an acute case of Cushing's syndrome Although insufficiently confirmed in patients with this disease, the thesis finds support in the animal experiments of Long and his associates (31) If true in Cushing's syndrome, it is conceivable that the increased appetite observed in the early stages of the disease represents an unsuccessful attempt at replacement of essential body protein and that despite the continued loss of body protein a positive *energy balance* exists with a resultant deposition of body fat That a dissociation of protein and energy balance may occur in the opposite direction, i e , a negative energy balance associated with positive nitrogen balance has been shown in our laboratory (32)

*Pancreatic islets* A relationship between hypoglycemia and the hunger mechanism has long been recognized It has been suggested that a mild degree of hypoglycemia, insufficient to produce typical symptoms, could be the cause of the desire of obese persons for increased amounts of food Studies of blood sugar levels in obese people fail to disclose any increased tendency toward hypoglycemia In fact, the trend is decidedly toward hyperglycemia (33)

Chronic, recurrent, spontaneous hypoglycemia in humans, severe enough to produce symptoms, may result from any one of a variety of causes (34) Obesity, however, is a prominent feature in only one of these disturbances, namely, organic hyperinsulinism In this condition hypoglycemia is likely to be more severe and more prolonged than in any of the other conditions which result in low levels of blood sugar The patient often learns that he can prevent or alleviate attacks by frequent ingestion of food Hence, some become severely obese But others with hypoglycemia of equal severity never experience an increased desire for food and do not become obese

Functional hyperinsulinism (34) represents a very frequent type of spontaneous hypoglycemia manifesting itself in the form of transient symptoms occurring two to four hours postprandially These individuals invariably maintain a normal level of fasting blood sugar No lesion of the islet cells is recognizable in this group, but it is suggested that functional hyper-responsiveness of the insulogenic mechanism accounts for the severe but transient hypoglycemia

demonstrable These individuals are of average weight or lean, are highstrung, emotionally unstable and display many other signs of instability of the vegetative nervous system We have studied a large number of such cases and are impressed by the virtual absence of obesity in this group

It appears, then, that if hypoglycemia is to play a significant rôle in stimulating *excessive ingestion of food, it must be severe and prolonged to the point of* easy clinical recognition That such a situation is absent in the great mass of obese individuals is obvious.

These clinical observations are borne out by the animal experiments of McKay and Calloway (35) and of Barnes and Keeton (36) It was found that rats could be made to ingest sufficient extra food to produce obesity only when severe hypoglycemia was maintained continuously by the use of large amounts of protamine zinc insulin Rapidly absorbed insulin which produced severe but relatively transient hypoglycemia did not result in increased total consumption of food It is, therefore, extremely doubtful that "relative hyperinsulinism" plays any part in the production of obesity, with the rare exception of the patient harboring a pancreatic insuloma

The possibility that insulin may stimulate conversion of carbohydrate to fat has been suggested on several occasions But even were this assumption proven to be true, such a metabolic process would have no influence upon the production of obesity For whether energy requirements are provided by ingested carbohydrate or by fat derived from ingested carbohydrate is inconsequential Caloric balance would remain the same in either case and further assumptions would be necessary in order to explain increase in weight

Finally, any possible rôle, in the etiology of obesity, of lipotropic substances such as choline and methionine (37) or the pancreatic lipocaic of Dragstedt (38) is beyond present knowledge and need only be mentioned as being related to the metabolism of fat.

*Thyroid* Little need be said regarding the relationship of hypothyroidism to the development of obesity It is clear to all that if the basal heat production is reduced by 40 per cent and, in addition, the daily spontaneous activity is diminished by the very nature of the disease, the total dissipation of energy and, consequently, the requirement for energy will be significantly reduced Such an individual will unquestionably gain weight if he continues to consume an amount of food which was formerly barely sufficient to maintain his weight Interestingly enough, however, and contrary to the usual clinical teaching, obesity is by no means a reliable index or a constant feature of hypothyroidism (19, 39, 40) For the majority of such patients the hunger mechanism does an excellent job of diminishing food intake to the newly acquired level of metabolism In those in whom such adjustment is less sensitive, either obesity or undernutrition may result

*Gonads* The response of the body weight to gonadectomy is extremely variable in animals and in humans Castration of domestic fowl has long been known to produce larger and fatter animals Castrate lambs and goats gain less weight than their controls (41, 42) Gonadectomized male or female rats

show no increase in body fat over their controls (43) In men castration results in obesity in some and in the "tall, lean castrate" in others Women are said to gain weight rapidly after the menopause That this is not true is well shown in the statistics compiled by Rony (44)

From a practical point of view obesity in association with hypogonadism occurs with greatest frequency in pre-adolescent boys and girls At this stage of life hypogonadism is normal and the obesity is correctly regarded as "exogenous" The advent of normal puberty and the development of normal secondary sex characteristics is again correctly taken to mean that the continued obesity is not related to gonadal function But pity the child who is fat and in whom the onset of puberty is delayed for several years! He is labelled as a case of "gonadal obesity", "pituitary obesity" or "adiposo-genital dystrophy" and placed in a category of endocrine cripples Fortunately, the erroneous diagnosis proves to be shortlived The majority of such patients sooner or later pass spontaneously through a delayed puberty period and become sexually mature The attendant redistribution of body fat may change the appearance to one which conforms more closely to the normal male or female but the excess weight remains unless the patient is underfed As mentioned above, the use of gonadotropic substances in such cases may promote sexual maturity, but does not affect excess weight (23, 24)

On the other hand, it has been our experience in these children that simple weight reduction by dietary means has resulted in the fairly prompt establishment of puberty This interesting phenomenon, i e , the rôle of obesity per se in influencing gonadal function in adults as well as in adolescents requires elucidation

Heredity versus environment Little in the way of positive information can be cited for the case of "hereditary obesity" in humans As stated earlier, the search for the fundamental cause or causes of obesity must no longer concern itself with whether the development of obesity is associated with an excessive intake of calories That part of the problem is settled! Obesity, regardless of any precipitating cause, cannot develop unless associated with a positive energy balance But the underlying mechanisms which drive the individual to ingest energy in excess of his needs remain elusive Does there exist a gene or gene complex which, through its influence upon the central nervous system or the endocrine system or by another mechanism, forces upon the stigmatized individual an irresistible desire for excess calories? The answer to this important question is not yet available

Statistical studies (45, 46) while in many respects suggestive of an hereditary fault, have not eliminated environmental influences as factors of prime importance in the acquisition of obesity as a familial characteristic Studies upon groups of identical twins simply emphasize the importance of environmental influences upon body weight The studies of Verchuer (47) and of Neuman (48) indicate that the average variation of body weight of identical twins is two to three times greater than the average variation of any of the other anthropologic measurements That the average variation in body weight of fraternal twins

is greater than that of identical twins (48) gives no information referable to the transmission of a gene capable of producing the *abnormal state* known as obesity

The important experiments of Danforth (49) proved conclusively that in a yellow strain of wild mice there exists a gene, probably associated with that imparting the yellow color, which regularly produces obesity, that the obesity begins after sexual maturity, and that the obesity is much more marked in the female than in the male harboring the same gene Experiments with this strain carried out in our laboratory, indicate that an increased consumption of food occurs in those animals which become obese Thus, in this species, there can be little doubt that heredity plays a dominant rôle in initiating a positive energy balance That a similar background may exist in some humans can at present be neither denied nor affirmed

On the other hand, the positive influence of environmental factors upon body weight is well established Almost all students of obesity agree upon one point, i e, that at least some obese individuals are victims of circumstance and that their obesity is the result solely of a perverted appetite Hence, all classifications of obesity include a type designated as "exogenous obesity" The experienced clinician, while justifiably reserving the right to consider hereditary factors in the etiology of obesity, has no doubt about the influence of early training acquired habits of food selection and emotion upon the development of the condition Appetite, as opposed to hunger, is a product of one's environment and is importantly affected by pleasurable and painful experiences One emotionally disturbed person is distressed by the sight or smell of food (anorexia nervosa) while another finds relief of mental anguish in the pleasurable sensation of a full stomach The direction of his reaction toward food may be significantly influenced by past experiences, training and habit This is not to imply that all or even a majority of obese people are emotionally upset Many are simply victims of early training They have been conditioned to a higher "satiety level" than is required by the physiological needs of the body

Anomalies of metabolism A number of theories have been proposed suggesting an abnormal metabolic pathway for fat, protein or carbohydrate, the result of which was to lead to the deposition and retention of excessive amounts of adipose tissue Devoid of experimental confirmation, such concepts arose because of the common clinical observation that many obese people subjected to stringent and controlled underfeeding fail to lose weight as expected The experiments dealing with the masking effect of temporary retention of water during periods of actual destruction of tissue (see Part I) soon dampened the enthusiasm of those who had contended that the fundamental laws of thermodynamics could be violated by certain fat people The more recent concepts of disordered metabolism as a cause of obesity have included the obvious necessity for excessive calories The abnormal metabolic process is then held responsible for the excessive desire for food Since some of these alleged metabolic aberrations have been dealt with in detail elsewhere the reader is referred to the previous discussions where possible

1 *Hypoglycemia* (See under Endocrine Obesity)

2 *Lipophilia* (See under Lipophilia, Part I)

An additional comment regarding lipophilia (fat-fixation, fat immobilization, etc ) is indicated at this point Bauer (50) although holding firmly to the concept of increased lipophilia as the important mechanism in the production of obesity, freely admits that restriction of calories is the only sensible and effective method of treatment The fact that any obese individual when fed a diet submaintenance in calories, but sufficiently adequate in protein to provide for nitrogen equilibrium, loses 50, 100 or more pounds of body weight, is in itself the strongest argument against immobilization of body fat as a source of fuel One cannot reconcile the concept of tissue lipophilia with the finding that body fat will provide the energy to make up whatever caloric deficit is provided by underfeeding

3 *Hypolipemia* Wilder (51) has suggested that a slightly greater than normal withdrawal of fat from the circulation postprandially could explain the delayed sense of satiety encountered in obese people and could account for the increased total intake of food Such a mechanism, if it existed, could explain the *maintenance* of obesity (once established) were the increased rate of withdrawal of circulating blood fat due to the increased need for energy known to be associated with the obese state But in order to be important as a *cause* of obesity, such an abnormality would have to be shown to exist in certain individuals before the onset of obesity, and to persist after successful dietary reduction of weight Such information is not available A point, therefore, to be evaluated with great care in interpreting any metabolic variant observed in obesity, is the effect of the obesity per se in producing the abnormality encountered

STATIC OBESITY Finally, any discussion of possible etiological factors involved in the production of obesity should bring out several significant points with regard to the maintenance of the obese state A critical analysis of static obesity helps to simplify the problem of its production

It is common knowledge that most obese people eventually reach a level of body weight which remains relatively constant thereafter The level may be 200 pounds or 500 pounds or any other amount which indicates the presence of obesity What are the energy relationships when this static weight is reached? Does the cessation of gain in weight mean that the individual is now eating less than he has been previously? Decidedly not! Assuming that his gain of weight has been steady and even, he will now be found to be eating at least as much as he was at any time during the period of increasing weight The only possibility remaining is that he is now expending more energy than had been the case during the time that his weight was increasing Let us examine the facts

That basal heat production rises in relation to increasing surface area is a classical and well established phenomenon This means that as the body enlarges, the requirement for ingested energy to make it grow larger increases progressively There comes a time when, because of increased size of the body an equilibrium is established between caloric inflow and caloric expenditure, and

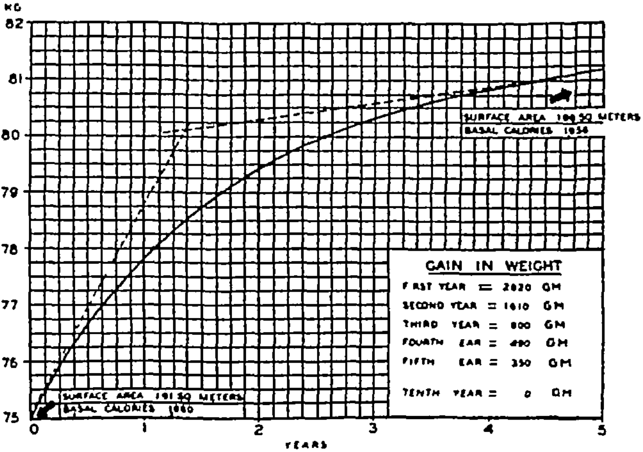


Fig 8 Weight plotted against time with an initial dietary excess of 80 calories per day The broken lines indicate the rate at which weight would be added in the first and fifth years

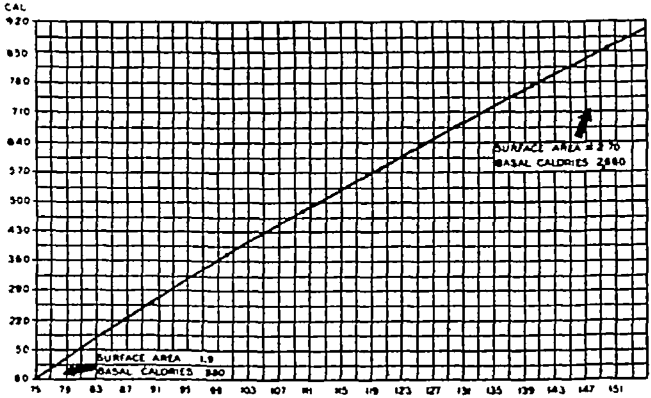


Fig 4 Excess calories plotted against weight In order to maintain a given rate of weight gain caloric intake must be increased progressively as the body enlarges

positive energy balance ceases Weight is then maintained at the new high level unless energy intake is further increased The amount of energy now

required for maintenance of body weight was formerly sufficient to produce at first a rapid and later a slow gain in weight Figures 3 and 4 are clearly illustrative of this phenomenon

This physiological increase in the dissipation of energy as surface area increases is not peculiar to any class of warm blooded animals (52) It occurs in any normal animal or human who is forced-fed to the point of increased surface area Thus the mechanisms for the *dissipation* of energy in the obese individual respond in the normal way One is faced, therefore, with the inevitable conclusion that the defect in obesity, whatever its dynamic mechanism, is to be found on intake side, i e , the ingestion of excessive amounts of energy

## SUMMARY

Obesity is invariably the result of an overall intake of energy which has exceeded the total dissipation of energy by the body, i e , a positive energy balance With the few exceptions associated with an abnormally low basal metabolic rate (and the latter is by no means limited to fat people), the mechanisms for the dissipation of energy from the obese body are normal The fundamental fault, therefore, is to be found in the cause or causes of an excessive desire for food

Experimentally a number of mechanisms leading to a desire for excessive amounts of food have been demonstrated They include bilateral hypothalamic injury (rat, dog and monkey), severe and prolonged insulin hypoglycemia (rat), and hereditary bulimia in a strain of mice It has not been established that these are significant causal factors in human obesity

Abnormalities of the endocrine system which affect adipose tissue are more importantly related to the distribution of body fat than to excessive accumulation of it The search for metabolic aberrations which could lead to obesity has not been fruitful

Environmental influences upon appetite are established causes of a very large number of obese patients These include emotional difficulties, family habit, early training, affluence, boredom, etc Other patients become obese, not because they eat more, but because they expend less Various types of prolonged illness, advancing years and change to less active occupations result in obesity when a corresponding diminution in appetite fails to take place

The classification of obesity as exogenous, endogenous, endocrine, pituitary, thyrogenous, gonadal, etc , is misleading and should be discontinued

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# THE CELLULAR COMPOSITION OF NORMAL BONE MARROW AS OBTAINED BY STERNAL PUNCTURE

EDWIN E OSGOOD AND ARTHUR J SEAMAN

*The Division of Experimental Medicine, Department of Medicine, University of Oregon Medical School, Portland*

A critical appraisal of the literature on the cellular constitution of the marrow of healthy individuals can be made only in the light of an understanding of the anatomy and physiology of the marrow, the objectives of marrow studies, the methods of study available, and the many variables affecting the composition of the specimen obtained for examination

ANATOMY AND PHYSIOLOGY OF BONE MARROW (1) The marrow is the largest, most widely dispersed, and least homogeneous organ in the body Its volume is one to two times that of the liver, or 1,600 to 3,000 cc in the adult Its location within the interstices of cancellous bone and between the trabeculae of the marrow cavities in long bones makes complete study of all the marrow an almost impossible feat The vascular and nerve supply are imperfectly understood, what knowledge we do have being based on studies on birds or lower mammals under abnormal conditions These studies suggest that sinusoids probably substitute for the capillary bed of most organs and that these sinusoids are probably a closed system with most of the sinusoids collapsed at any one time Human bone marrow varies from an almost exclusively fatty tissue to the most cellular red marrow, with all gradations in between While in the adult fat predominates in the shafts of the long bones and red marrow in the cancellous heads of these bones and the flat bones, all gradations are often seen in a single histologic section of grossly red marrow from a healthy person Not only is cellular marrow interspersed with fat but in the cellular area adjacent foci consist of different cell types and lie next to vascular channels filled with blood In disease this patchy structure is still more pronounced, as witness the lesions of multiple myeloma, metastatic neoplasms, and the lipoid histiocytoses

The important functions of the marrow are the formation of the cells of the blood, the removal of particulate matter from the blood, and the possible production of antibodies and plasma proteins Except in disease or embryonic life all formation of cells of the erythrocytic, granulocytic, and thrombocytic series apparently occurs in the marrow, and the formation of cells of the lymphocytic, monocytic, and plasmacytic series is shared with the lymph nodes, spleen, and other lymphoid or reticuloendothelial tissue Evidence suggests that erythrocyte formation is primarily intravascular in temporarily closed sinusoids and that the other cells are formed extravascularly

The formation of these cells involves the fundamental processes of growth increase in size or hypertrophy, either mitotic or amitotic cell division or hyperplasia, maturation or differentiation, and, in addition, release into the circulation In the formation of platelets the splitting off of fragments of cytoplasm is also important The important cells vary in diameter from one or two micra for the smallest platelets to sixty micra or more for the megalokaryocytes

OBJECTIVES OF OBTAINING MARROW SPECIMENS Marrow specimens are studied to learn more about its structure and its function in health, that is, to add to knowledge of normal anatomy and physiology, to detect deviations from normal in disease for diagnostic purposes, or to obtain material for tissue culture, for bacterial culture, or for identification of parasites The method of choice depends upon the objective.

METHODS OF OBTAINING MARROW SPECIMENS, WITH ADVANTAGES AND DISADVANTAGES OF EACH METHOD The ideal method of obtaining marrow specimens would permit removal of a large, representative, measured specimen in such form that accurate cell identification and counts of each type of cell may be obtained and all structural relationships could be observed. Unfortunately, no such method exists The methods which are available include postmortem sections, procurement of a button of marrow by use of a trephine during life, and aspiration of marrow through a needle, either postmortem or during life, and, in conjunction with the latter method, the possible removal of a core of marrow by the use of a serrated hollow drill longer than the needle (2) Each method has certain advantages and certain disadvantages

*Postmortem study of marrow is probably best for determining the normal anatomy of the marrow* It permits sections of all the major bones, so that a gross determination of the relative distribution of yellow and red marrow may be made Gross inspection of the cross sections of bone may guide the selection of the tissue to be removed for microscopic study Numerous enough and large enough specimens may be obtained to discount the patchy distribution of cells within the marrow As with the other methods, fixed sections, imprints, and cell suspensions may all be studied, and special injection technics through the vascular system may be employed There are, however, many disadvantages to postmortem studies A diagnosis made then is obviously too late to be of value to the patient Unless the specimens are obtained within a few minutes after death autolysis will have set in (3), cell morphology is destroyed, and coagulation of the blood prevents aspiration of a satisfactory sample Only rarely is postmortem material available within a few minutes of death when there is adequate evidence of the previous good health of the subject The few studies so far made (4) on such material have been inadequate in one or more respects Not enough subjects have been examined Criteria of previous health have been inadequate Gross and microscopic sections have not included enough bones Imprints and cell suspensions have usually not been made Yet it appears that accurate data on the actual normal anatomy of human marrow can only be obtained by postmortem study It is important when such a study is made that measured or weighed blocks of marrow of one or more cubic centimeters volume be suspended in measured volumes of homologous serum and that total nucleated cell counts as well as differential cell counts on smears from this material be compared with aspirated marrow from an adjacent area and with sections and imprint preparations The volume and morphology of the unsuspendable residue should also be noted

Obtaining a button of marrow by trephine during life (5) has the advantages of permitting the ready preparation of imprints and microscopic sectior

several serious disadvantages, however It leaves a disfiguring scar which is especially objectionable to women It is a more formidable procedure than aspiration So small an area is examined that patchy lesions are easily missed A total nucleated cell count and an accurate differential cell count are impossible, as will be shown later Although Dameshek, Henstall and Valentine (6), from their study of the comparative quality of preparations made from material obtained by trephination and by sternal aspiration from the same patient, concluded that the cellularity, reticulocyte percentage, relative number of erythroblastic to granulocytic cells, and absolute number of early nucleated cells in smears made from material obtained by trephination was much greater, Falconer and Leonard (7), in a comparable study, after evaluation of preparations made by simultaneous trephination and sternal aspiration on 13 cases state "In only one of these were significantly superior preparations as to number, type, and distribution of cells obtained by the trephine method "

Needle aspiration of marrow has, since the introduction of the method by Caronia (8) in 1922 from the tibia in children and by Arinkin (9) in 1927 from the sternum in adults, shown such definite advantages for diagnostic purposes that it is now used all over the world It is a simple procedure which can be done in the office or at the bedside Cell morphology is better than with any other method Total nucleated cell counts as well as differential cell counts are possible Enough material is obtainable for bacterial (10) and tissue (11) culture, peroxidase and reticulocyte stains, thick drop preparations, or other special procedures, and to permit diagnosis of patchy lesions such as multiple myeloma and lipoid histiocytoses, as well as the diffuse lesions of leukemias, anemias, marrow aplasia, malaria, and leishmaniasis If imprints and sections are desired small fragments of marrow may usually be fished from the punctate from which satisfactory preparations may be made (12), or a hollow drill with serrated edge (2) may be inserted through the needle to obtain a core of marrow before aspiration The disadvantages of the method are that patchy lesions will occasionally be missed and that there may occasionally be failures to obtain marrow One of the authors (E E O ) has failed to obtain marrow only once in the last thousand punctures, but marrow was obtained from this patient on another occasion There were 5 failures among the first 62 sternal punctures done (13) It seems certain, however, that failure to obtain marrow would result in cases of osteosclerosis The criticism often raised (14) that cell relationships are lost and that a variable amount of blood is aspirated is valid if only the suspension is examined, but as pointed out above material suitable for sections and imprints can usually be obtained In the majority of instances aspirated material is satisfactory for diagnosis, if a diagnosis can be made by examination of the marrow obtained by any technic, although x-ray studies and necropsy marrow examination are unquestionably more likely to detect osteomyelitis and metastases of malignant tumors

THE RELATIVE MERITS OF SECTIONS, IMPRINTS, AND SMEARS FROM CELL SUSPENSIONS Sections, imprints, or smears from cell suspensions can be made from material obtained by any of the above methods

Microscopic sections have the advantage of preserving cell relationships and of including all types of cells Advocates of this method claim to be able to identify cells in such sections, and some (4) even report differential cell counts on sections However, even if all the cells and cut fragments of cells could be identified in a section, which the authors doubt, differential cell counts would be grossly in error, for the reason that the larger the cell the more times fragments of such cells would appear in the section, and the smaller the cell the more likely is it to be obscured by an overlying cell or fragment of cell When the finest nuclear detail is often necessary for identification, how can the cell fragments from which most or all of the nucleus has been cut off be identified, and how is one to decide how large a fragment of a cell must appear in a section for it to be counted?

Imprint or touch preparations made from the freshly obtained buttons of marrow obtained by trephine during life or within a few minutes after death have the advantages of giving excellent cell morphology in the thinner areas and of preserving the general grouping of cells in the marrow The inclusion of cell fragments and the understaining of thicker masses of cells which adhere to the slide make it impossible, however, to do a systematic differential cell count of all cells in the imprint, and the probable differences in the adhesiveness of various types of cells make it very unlikely that the proportions of cells adherent to the slide actually represent the proportions of cells in the marrow Furthermore, because of the tendency of similar cell series to appear in groups in the marrow an accurate differential cell count is impossible, even if all cells were identifiable, unless extremely large numbers were counted, because of the chance selection of patches of cells of predominantly one type

Cell suspensions obtained by the technic of sternal marrow aspiration have the great advantage that they may be thoroughly shaken, insuring a uniform distribution of the cells so obtained, of permitting quantitative total nucleated cell counts which give a rough idea of the degree of cellularity of the marrow, and of permitting accurate cell identification and differential cell counting The proportion of mechanically traumatized or damaged cells is much less than in the other methods The disadvantages are that cell relationships are lost, the cells may not be equally easily aspirated so that the proportions of cells may not be that actually occurring in the marrow, and that the amount of dilution with blood and plasma may not be accurately determinable The fat cells, fibroblasts and reticuloendothelial cells known to be present in the marrow are not usually present in the aspirated material in identifiable forms That these disadvantages are not serious is demonstrated by the fact that correct diagnoses are usually achieved by examination of such material The cell relationships which existed in the marrow can usually be accurately predicted from the cell types found in the punctate, the relative percentages, and the total nucleated cell count, if in the interpretation the existant knowledge of the gross and microscopic cellular distribution in the corresponding disease is considered for example, the finding of Gaucher cells, tumor cells or a moderate increase in plasmacytes indicates the presence of the patchy lesions of Gaucher's disease, metastatic malignant tumor or multiple myeloma, whereas, the great increase in the type cell of any

form of leukemia or the immature nucleated erythrocytes indicates the diffuse hyperplasia characteristic of the corresponding leukemia or anemia of the variety indicated by the cell morphology The nucleated cell count shows a high correlation with the proportion of fatty or fibrous tissue to hematopoietic cells, so that low total nucleated cell counts indicate aplasia, agranulocytosis, or osteosclerosis Occasionally, in certain aleukemic leukemias and when the needle enters the metastasis of a malignant tumor, the cells are so firmly adherent to one another that a representative number is not aspirated and the total nucleated cell count is low Usually, however, a sufficient number of the characteristic cells of aleukemic leukemia is aspirated to establish the correct diagnosis, and occasionally this is true in malignant tumors In the presence of metastases of malignant neoplasms, other methods are more reliable than any form of marrow examination A high nucleated cell count indicates hyperplasia, except in those cases when a very small quantity of marrow is aspirated and the tip of the needle lies in a nest of cells or when the proportionately larger evaporation in relation to the small volume is not prevented These possibilities are eliminated by taking a larger amount The variable dilution with blood and plasma applies also to sections and imprints It must be remembered that blood is present in the sinusoids and vascular channels in both normal and pathologic marrows, and is of necessity included in such preparations as well as in preparations made by the technic of aspiration

It is evident, therefore, that the method of choice for clinical diagnosis or for obtaining marrow for bacterial or tissue culture is the aspiration and adequate examination of sternal marrow, and that for the study of the normal anatomy the method of choice is the procurement and adequate examination of fresh necropsy material from healthy persons who were executed or killed accidentally or in battle

THE SITE OF PUNCTURE It is possible to obtain marrow by needle puncture aspiration from many different bones, including the shaft or head of the tibia, the head of the femur, the crest of the ilium, the body or manubrium of the sternum, the ribs, and, according to Nordenson (15), the vertebrae Since in adults it is known that the marrow from the shafts of the long bones is predominantly fatty it would seem desirable not to use this site in a study of the normal marrow picture In infants and young children this marrow is deeply red, and considerable work has been published on the tibial marrow in children (16) Obviously, any study of marrow obtained by biopsy should state the exact site of puncture A number of different sites have been used by different authors There is considerable evidence that the marrow obtained by aspiration from different areas in the same bones or from different bones in the same person is of similar composition, if the person is healthy and the sites are those that normally show red marrow Nordenson (15) compared the differential cell counts on sternal marrow with those taken from the vertebra, rib, pelvic bone, and tibial epiphysis in two patients with normal peripheral blood pictures and found good agreement in all the areas except the latter which was almost completely inactive Stasney and Higgins (17) compared the marrow obtained within five hours after accidental death from 14

persons without evidence of disease of any hematopoietic organ or signs of infection They examined sternal marrow, rib marrow, and vertebral marrow and found excellent agreement in the proportions of the granulocytic and nucleated erythrocytic series and in the differential cell counts Yamamoto (18), Lossen (19) and Williams (20) each reported that there was a marked similarity in the cellular pattern of marrow taken from different regions Conclusions to the contrary by Helpap (21) were based largely on gross pathologic findings and not on actual comparative cell counts

Since the majority of the work has been done on the sternum and since it is the most convenient site accessible for puncture it would seem best to standardize on the sternum as the site of choice, except when local tumors demonstrated by physical examination or radiography indicate a reason for puncture at another site

Greif (22), comparing different sites of puncture in the sternum, found excellent agreement between neighboring punctures at the same horizontal level and not quite as good agreement between punctures at different vertical levels, although as will be mentioned later his method may introduce considerable error A number of authors prefer the manubrium to the body of the sternum Veeneklaas (23), from anatomical studies of fifty sternums in infants, recommended the manubrium of the sternum as the site of choice for puncture in this age group Diwany (24), from x ray and postmortem studies, also recommends that the manubrium be used for the first few months but prefers the first sternebra in infants over six months of age

Since sternal puncture is more easily performed through the sternomanubrial junction because the soft cartilaginous end of the bone is easily penetrated by rotating the needle with very little pressure, obviating the use of a hammer or mallet to drive in the needle, and since this would standardize the level of puncture, it is recommended that the midline of the sternomanubrial junction should be the site of choice for introduction of the needle It makes little difference whether it is directed into the body of the sternum or into the manubrium This choice may be based on the anatomic structure of the particular subject The body of the sternum is usually preferable in adults and the manubrium in children under six months of age or in those adults whose manubria project anterior to the surface of the body of the sternum

THE TECHNIC OF PUNCTURE Many different methods of sternal puncture have been described, but it is not in the province of this article to review these methods Certainly, however, an article on normal values should state the exact method employed The methods vary in the size of the needle, the direction in which it is inserted, the site of puncture, and particularly in the method of forcing the needle into the marrow cavity One investigator (25) used a mallet or hammer to drive in the needle Often a guard which is supposed to prevent too deep penetration by the needle is utilized (26) While any of the methods described is apparently satisfactory as far as obtaining material suitable for diagnosis is concerned it would seem that the simplest and safest technic producing the least discomfort for the patient should be the method of choice The simplest and

safest method (27) so far available seems to us to be that one in which, after adequate local anesthesia of the skin and periosteum, a 16 to 18 gauge needle with a well fitted stylet and a knurled head is introduced diagonally through the sternomanubrial junction and rotated without much pressure in one direction like a drill, at all times under perfect control by the fingers of the other hand resting on the sternum It certainly requires the least complicated and expensive apparatus

THE AMOUNT OF MATERIAL NECESSARY The amount of material aspirated obviously will have a considerable effect on the number of marrow-specific cells and on the distance from the needle from which they can be obtained, yet some authors fail to mention the amount of marrow aspirated (28) Greif (29) found that if 0 1 cc portions were aspirated successively and examined separately, whether the first 0 1 cc was aspirated quickly and the second slowly or vice versa, the first 0 1 cc had a consistently higher total nucleated cell count than the second 0 1 cc He, therefore, recommended that the first 0 1 cc be used for examination In 1938 (22) he aspirated and made total nucleated cell counts on successive drops up to 5 drops on 15 cases A consistent tendency for the total nucleated cell count to decrease in the first three drops and then to level off was noted He also compared the first 0 1 cc with the second 0 1 cc aspirated on 15 cases and noted a lower count in the latter than in the former Segerdahl (30) compared successive 0 2 cc aspirations and found a considerable decrease in count in the second 0 2 cc Reich and Kolb (31), on marrows from patients with various diseases, compared the first 2 5 cc aspirated with the second 2 5 cc aspirated Inspection of their figures reveals no consistent tendency for the second count to be lower or higher than the first count, and the majority of the counts agreed within the limits of error of cell counting technic, although the authors themselves conclude that "statistical analysis indicates that quantitative determinations on aspirated marrow samples are inaccurate " One of us (E E O ) has made a few unpublished studies, comparing the first 0 1 cc , the first 1 cc , and the last 1 cc of 10 cc samples aspirated A considerably higher count was noted in the first 0 1 cc than in the 1 cc sample, but the first 1 cc and the last 1 cc of 10 cc aspirated showed comparatively good agreement, considering the unavoidable errors in cell counting technic There was no consistent tendency for the last 1 cc to have a lower count than the first 1 cc

On the basis of the higher counts in the first 0 1 cc , many authors have felt that it was wiser to do all the work with the first 0 1 cc aspirated from the marrow (32) However, for practical clinical purposes there are several objections to this It is difficult to measure accurately such a small volume, and the fact that there is great change in cell counts in successive 0 1 cc portions aspirated means that considerable errors will be produced from this cause alone Removal of such a small quantity can not possibly aspirate cells from more than a few millimeters from the tip of the needle, whereas, if 1 cc or more is aspirated cells from a considerable distance might be included, thus obtaining type cells from patchy lesions which the needle did not actually penetrate During the process of transfer of a small volume, such as 0 1 cc , to a watch glass

mixing, and reaspiration for counts and making smears, very appreciable loss by evaporation must take place, and this will be variable with the time consumed and the temperature and humidity of the room Since, when larger volumes are aspirated the count no longer becomes progressively lower but tends to remain uniform or show plus or minus variations, it is obvious that one is not continuing to draw only blood and further dilute the sample but to withdraw a more uniform mixture of blood and marrow Inspection of table 1, in which total nucleated cell counts are summarized, also reveals that where larger amounts were taken the total variability of range was markedly decreased. Part of the variability when small samples are taken is undoubtedly due to the chance location of the tip of the needle near a nest of cellular marrow or within a blood sinus or in a less cellular fatty area The larger samples also permit use of a measured amount of anticoagulant in relation to the amount of marrow fluid aspirated and examination over a period of time with a chance to do additional tests, such as a peroxidase stain, multiple smears, a reticulocyte count

TABLE 1

*Total nucleated cell counts per cubic millimeter of sternal marrow in adults*

| AUTHOR | NO OF CASES | CC. ASPIRATED | AVERAGE | RANGE |
|---|---|---|---|---|
| Tempka and Braun (28) | 3 | | 55 200 | |
| Segerdahl (30) | 110 | 0 2 | 77 194 | 10 600–238,000 |
| Napier and Gupta (63) | 10 | 2 0 | 53 500 | 32 500–116 000 |
| de Renzi and Fuortes (28) | 20 | | 33 115 | |
| Pitts and Packham (64) | 24 | 2 0 | 28,100 | 7 700– 46 000 |
| Gormsen (65) | 30 | 0 1–0 2 | 64 000 | 18 000–216 000 |

etc., should the routine total nucleated cell count and differential cell count indicate that such additional examinations are necessary

It is recommended, therefore, that for diagnostic purposes 1 0 cc or more of marrow be aspirated and that if the actual cellular constitution of marrow as it exists is to be determined, fragments of marrow, after measurement of the volume, be suspended in a known volume of homologous serum and counts be made on these or from a core of material removed with a hollow drill with a serrated edge and similarly suspended If the smaller quantities are to be used for diagnostic purposes it will certainly be necessary for the hematologist always to remove exactly the same amount of marrow in the same manner, to make a correction for evaporation based on the temperature, humidity, and time of making preparations from that marrow, and to determine his own normals for that particular technic

PREPARATION OF MARROW FOR EXAMINATION Great variations in the methods of preparing marrow for examination exist Some authors examine smears or imprints of solid fragments of marrow (33) Some allow the material to clot and make tissue sections (34) Others centrifugate and make smears and sections from the buffy coat (35) A few, including Greif (22), Joppich and

cytes) by other authors and in the rhabdocyte (staff cell) column by still others There was also a wide difference in the line of separation between the neutrophil rhabdocytes (staff cells) and neutrophil lobocytes (polymorphonuclear neutrophil leukocytes) Some authors divided them on the basis of whether they were filamented or nonfilamented, which we recommend, and others segregated the lobocytes (polymorphonuclear neutrophil leukocytes) from the rhabdocytes (staff cells) when there was a slight indentation of the nucleus Because of the relatively small numbers it did not seem practical to include the different stages of development of the eosinophils or of the basophils separately Notwithstanding the grouping of all forms of eosinophils together there is a surprising variation in results If any cell should be easily recognized and identified as the same by all investigators they would certainly seem to be the eosinophils The high mean value of 9 1 per cent eosinophils was in Indian subjects, which makes one suspect that parasitic infestation may have been present

Criteria for classification of nucleated erythrocytes were even more variable and difficult to interpret than those given for the leukocytes This difficulty has been discussed elsewhere (59), so it will not be repeated here, except to emphasize that different authors (46) use the term "megaloblast" with entirely different meanings which are mutually exclusive

The miscellaneous column includes a number of different types of cells that could not justifiably be placed under any other heading The names used by the original authors for these cells will be found beneath the table with the corresponding capital letter Young and Osgood, and Pitts and Packham included disintegrated cells in the differential cell count, and allowance must be made for the resulting reduction in the percentages of the other cell types Megalokaryocytes are too few in number to be counted accurately in the ordinary differential cell count Limarzi and Schleicher (60), by another method, have estimated the number of these cells as 58 8 per million nucleated bone marrow cells, or approximately 0 006 per cent Because Morrison and Samwick, in their original article, gave percentages for the granulocytic series and the nucleated erythrocytic series, totalling 100 per cent for each series it was necessary to recalculate their data from the ratios of the granulocytic series to the nucleated erythrocytic series, or the granulocytic series to the lymphocytic series, and of the granulocytic series to the plasmacytic series which were given, and the 3 0 per cent in the miscellaneous column equals the error in recalculation to a basis of a differential cell count of all nucleated cells equal to 100 per cent The authors agree with Segerdahl (30) and Naegeli (61) that the category of Ferrata cells should probably be discarded, since they apparently represent disintegrated granulocytes (myelocytes), progranulocytes (promyelocytes), and granuloblasts (myeloblasts), and occasionally disintegrating promonocytes from the descriptions and illustrations given (62)

Although the series is obviously not homogeneous enough in criteria of cell identification to permit accurate interpretation of the means, it did seem worth while to calculate the weighted means of the cell groups In making these calculations it was necessary to group together certain categories because of

much overlapping The range of means is also included to give an idea of the wide differences obtained by different authors This seems to be due largely to differences in cell identification The weighted means of the total granulocytic series, the lymphocytic series, the monocytic series, and the total nucleated erythrocytic series were also calculated so that the ratios could be computed The ratio for the entire series of cases in table 2 of granulocytic series nucleated erythrocytic series was 63 0 17 21, or 3 67 1 0, of the granulocytic series lymphocytic series was 63 1 14.2, or 4 44 1 0, of the granulocytic series mono cytic series was 63 21 1 71, or 37 1

Segerdahl (30) was the only author who studied men and women of comparable ages, and she found no significant differences in the values for the granulocytic series and very slightly lower values for the nucleated erythrocytic series in women Some idea of the similarities of results in the two sexes in similar age groups can be obtained by comparing the data of Young and Osgood with those of Pitts and Packham These studies were made by the same technic with identical criteria for cell identification The former studied only males, and the latter studied only females

The data available on differential cell counts in infants and children are tabulated in a similar manner in table 4 The tabulation was made in order of increasing age from premature infants to children up to 15 years of age The averages were not computed, since age groups obviously differed greatly From a comparison of Tables 3 and 4 it is apparent that the differences in differential cell counts in children and adults are relatively slight compared to the variations in the figures of different investigators on the same age group If there is any significant difference it is a slightly higher percentage of granuloblasts (myeloblasts) and progranulocytes (promyelocytes) and a slightly lower percentage of neutrophil lobocytes (polymorphonuclear neutrophil leukocytes) in children

COMMENT It was hoped, when this review was undertaken, that from a compilation of the data available specific recommendations could be made as to normal standards for aspirated marrow It is apparent from the data presented that this must await further studies and a more general agreement on the technic of marrow aspiration and the handling of the material after aspiration, and especially on the acceptance of more uniform criteria of cell identification and nomenclature.

Until such time as further data are available it is suggested that investigators use the figures in table 5 as tentative normal standards The figures for the larger amounts aspirated were calculated from the data of Napier and Gupta (63) and Pitts and Packham (64) for total nucleated cell counts, and from the data of Young and Osgood (13) and Pitts and Packham (64) for differential cell counts These figures should be used if 0 5 to 10 cc of marrow is aspirated and disintegrated cells are included in the differential cell count, as is recom mended The figures for the smaller amounts aspirated were calculated from the data of Segerdahl (30), and should be used if the investigator uses her technic and criteria of cell identification, and prefers to omit disintegrated cells and to aspirate 0.2 cc. or less of marrow, notwithstanding the disadvantages which

TABLE 4

*Differential cell counts reported on sternal marrow of healthy infants and children*

| AUTHOR | NO OF CASES | AGE | GRANULO-BLASTS | PROGRANULO-CYTES A | PROGRANULO-CYTES S | GRANULO-CYTES | META-GRANULO-CYTES | RHABDO-CYTES | LOBO-CYTES | EOSINOPHILS | BASOPHILS | LYMPHO-CYTES | MONOCYTES | NUCLEATED RBC | MISCEL-LA-NEOUS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ichtenstein and Nordenson | 17 | Premature | 3 98[a] | 4 18[b] | ( | 14 36[c]) | 7 67[d] | 4 20[f] | 15 51[g] | 2 4 | 0 0 | 13 8 | 1 97 | 16 92 | 5 76[A] |
| (16) | 8 | Newborn | 4 0[a] | 3 66[b] | ( | 12 5[c] | 16 09[d] | 7 34[f] | 17 72[g] | 2 22 | 0 0 | 15 53 | 4 12 | 14 24 | 1 18[B] |
| hapiro and Bassen (66) | 35 | Newborn–24 hrs | 0 8[a] | ( | | 16 3[c]) | (33 9[h] | ) | 7 0[g] | 2 6 | 0 0 | 3 8 | 0 0 | 31 9 | 3 9[C] |
| | | 7 days | 1 5[a] | ( | | 19 7[c]) | (43 5[h] | ) | 10 5[g] | 2 3 | 0 0 | 6 2 | 0 0 | 11 6 | 5 0[D] |
| eeneklaas (23) | 3 | Under 14 days | 2 0[a] | 2 4[b] | ( | 13 2[c]) | 8 2[d] | 24 1[f] | 12 9[g] | 4 0 | 0 0 | 19 0[i] | 0 0 | 12 0 | 2 8[E] |
| ogel and Bassen (56) | 12 | Under 24 days | 1 94[a] | 0 21[b] | ( | 21 86[c] | 24 55[h] | ) | 13 6[g] | 2 51 | 0 0 | 10 5 | 0 0 | 19 75 | 4 95[F] |
| ato (73) | 18 | 1–12 months | 2 0[j] | 3 1[k] | 7 2[l] | 9 4[c] | 11 8[d] | 12 6[f] | 4 5[g] | 5 6 | 0 0 | 17 4 | 0 5 | 26 0 | 0 0 |
| eenoklaas (23) | 65 | 14 days to 3 yrs | 2 5[a] | 2 4[b] | ( | 9 8[c]) | 10 0[d] | 10 4[f] | 8 6[g] | 1 35 | 0 0 | 36 7 | 0 6 | 13 3 | 1 0[G] |
| iwany (24) | 5 | 15 mos to 2 yrs | 1 6[a] | 0 85[b] | ( | 17 0[c]) | 16 6[d] | 6 4[f] | 7 0[g] | 4 8 | 0 0 | 27 7 | 0 05 | 19 4 | 0 0 |
| ato (73) | 32 | 1–15 years | 2 1[j] | 3 1[k] | 5 6[l] | 10 3[c] | 10 9[d] | 17 3[f] | 6 5[g] | 5 6 | 0 4 | 17 3 | 1 6 | 19 3 | 0 0 |
| acobsen (43) | 13 | 2–9 years | 1 26[a] | 2 83[b] | ( | 24 86[c]) | 21 02[d] | 4 46[f] | 8 3[g] | 4 14 | 0 19 | 18 22 | 0 98 | 12 47 | 1 26[H] |
| ogel and Bassen (56) | 29 | 2–12 years | 1 93[a] | 0 49[b] | ( | 17 1[c] ) | (24 79[h] | ) | 15 16[g] | 3 95 | 0 06 | 7 9 | 0 0 | 23 04 | 5 15[I] |
| eeneklaas (23) | 33 | over 3 yrs | 1 5[a] | 2 2[b] | ( | 9 4[c]) | 11 5[d] | 13 2[f] | 14 4[g] | 1 6 | 0 0 | 26 0 | 0 6 | 14 0 | 2 2[J] |
| iwany (24) | 10 | 3½–5 yrs 5 mos | 0 88[a] | 0 48[b] | ( | 17 1[c]) | 20 3[d] | 12 0[f] | 10 5[g] | 6 67 | 0 0 | 19 9 | 0 4 | 11 5 | 0 0 |

[a]myeloblast, [b]promyelocyte with azur granules, [c]N myelocyte, [d]N metamyelocyte, [f]rod or staff forms, N , [g]N segmented neutrophils, [h]N non-segmented neutrophils, [i]lymphoblasts and lymphocytes, [j]myeloblasts (Totipotent stem cells), [k]myeloblastic leukoblast ,[l]promyelocyte with specific granules

[A]0 53 unclassified, 0 59 reticulum cells, 0 06 megakaryocytes [B]0 59 unclassified, 4 58 reticulum cells, 0 24 plasma cells, 0 35 megakaryocytes [C]3 8 hematagones, 0 1 megakaryocytes [D]0 1 megakaryocyte, 4 8 hematagone, 0 1 reticulum cells [E]0 2 megakaryocyte, 4 65 hematagones, 0 1 reticulum cells [F]0 1 megakaryocytes, 5 0 hematagones, 0 05 reticulum cells [G]0 3 megakaryocytes, 0 8 reticulum cells, 0 2 plasma cells, 1 5 Ferrata cells [H]0 3 megakaryocytes, 0 5 reticulum cells, 0 5 plasma cells, 0 3 Ferrata cells [I]0 4 megakaryocytes, 0 8 reticulum cells, 0 6 plasma cells, 0 4 Ferrata cells [J]0 9 reticulum cells, 0 13 plasma cells, 0 05 Ferrata cells

have been pointed out The weighted means have been smoothed and the 95 per cent ranges so calculated as to include approximately plus or minus two standard deviations The data on differential cell counts of Napier and Gupta (63) were not used in the calculation of the differential cell counts because of the omission of disintegrated cells and of their high eosinophil values which are probably more frequently encountered in residents of India than in residents of Europe and the western hemisphere

TABLE 5

*Tentative recommended standards*

| | 0.5 TO 10.0 CC. ASPIRATED* | | 0.05 TO 0.5 CC ASPIRATED† | | |
|---|---|---|---|---|---|
| | Weighted means | 95% Range | Weighted Means | 95% Range | |
| Total nucleated cell count | 35 000 | 10 000–100 000 | 75 000 | 10 000–160 000 | |
| Granuloblasts | 0 40 | 0 0– 1 0 | 1 25 | 0 0– 2 4 | Myeloblasts |
| Progranulocytes A | 1 4 | 0 0– 3 0 | 1 50 | 0 0– 3 0 | Promyelocytes |
| Progranulocytes S | 1 0 | 0 0– 3 0 | | | |
| Granulocytes | 3 2 | 0 0– 9 0 | 15 0 | 8 0–22 0 | N myelocytes |
| Metagranulocytes | 6 5 | 3 0–10 0 | 15 5 | 8 0–23 0 | N metamyelocytes |
| Rhabdocytes | 24 0 | 17 0–33 0 | 9 0 | 6 0–12 0 | N staff cells |
| Lobocytes | 15 0 | 5 0–25 0 | 22 0 | 15 0–28 0 | Polymorphonuclears |
| Eosinophils | 2 0 | 0 0– 4 0 | 3 0 | 1 0– 5 0 | Eosinophils |
| Basophils | 0 2 | 0 0– 0 5 | 0 2 | 0 0– 0 4 | Basophils |
| Lymphocytes | 14 0 | 3 0–25 0 | 18 5 | 7 0–30 0 | Lymphocytes |
| Monocytes | 2 0 | 0 0– 4 0 | 2 0 | 0 0– 4 0 | Monocytes |
| Karyoblasts | 0 2 | 0 0– 1 0 | | | |
| Prokaryocytes | 2 0 | 0 0– 4 0 | | | |
| | | | 12 0 | 3 0–21 0 | Erythroblasts |
| Karyocytes | 6 0 | 4 0– 8 0 | | | |
| Metakaryocytes | 3 0 | 1 0– 5 0 | | | |
| Disintegrated cells | 19 0 | 10 0–30 0 | | | |

* The smoothed weighted mean and the 95 per cent range of the total nucleated cell count were calculated from the data of Napier and Gupta (63) and Pitts and Packham (64), and those of differential cell counts were calculated from the data of Young and Osgood (13) and Pitts and Packham (64)

† The smoothed weighed means and 95 per cent ranges of the total nucleated cell count and the differential cell counts were calculated from the data of Segerdahl (30)

Unquestionably the most important suggestions that are justified on the basis of this review are that further studies of the cytologic composition of marrow obtained by sternal aspiration are urgently needed, as well as further studies on necropsy material obtained within a few minutes after death It is suggested that when such studies are made the following conditions should be fulfilled and the data included in the article adequate evidence of the health of the subject or, if necropsy material is used, of the health of the subject prior to death, the age, sex, and race of the subjects, the geographic location and the

altitude at the locale of the study, the number of subjects studied, the site and technic of the puncture, the amount of material aspirated and its preparation for study, the total nucleated cell count on a thoroughly mixed specimen with the number of cells actually counted, the methods used in making and staining smears, differential cell counts which must be done on the thin areas of the smears where the erythrocytes do not touch each other to avoid errors in identification, the total number of cells counted in the differential cell count, and, of especial importance, the criteria of cell identification, preferably with a reference to some readily available atlas in which all types of cells are illustrated, at least until some international standard of cell identification and nomenclature is agreed upon It is far easier to evaluate results if the actual figures on each subject as well as the averages and statistical constants are given

The true normal anatomy of human marrow will not be known until comparative studies by all the methods available, as outlined in the body of this article, are made on fresh necropsy material from the bodies of previously healthy persons who have died from sudden traumatic causes

Notwithstanding the limitations which have been pointed out and the lack, as yet, of accurate normal standards, the widespread use of sternal puncture is a testimonial to its value in the differential diagnosis of aleukemic and subleukemic leukemias, aplastic anemia, other puzzling anemias, malaria, leishmaniasis, multiple myeloma, and the lipoid histiocytoses, and to the value of bacteriologic cultures of marrow in subacute bacterial endocarditis and other bacteriemias

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# CHEMICAL METHODS FOR THE DETERMINATION OF DEATH BY DROWNING

ALAN R MORITZ

*Department of Legal Medicine, Harvard Medical School, Boston*

If drowning be defined as suffocation caused by the inhalation of fluid, the potential diagnostic significance of two categories of postmortem chemical findings should be considered One includes the changes that might be expected to result from obstructive asphyxia The other includes the changes that might be expected to result from an exchange of water or electrolytes between the inhaled fluid and the blood of the pulmonary capillaries

It is obvious that the evidence derived from chemical investigation of postmortem material will be of limited value in determining whether or not death has resulted from obstructive asphyxia Most of the chemical alterations that would constitute evidence of asphyxia if recognized during life are likely to be simulated by agonal or masked by postmortem change

Chemical evidence that asphyxia is the primary cause of death in drowning was first demonstrated experimentally by Brouardel whose findings were subsequently confirmed and extended by Lougheed, Janes and Hall It was observed by the latter that the oxygen saturation of arterial blood drops to approximately 15 volumes per cent during the first few minutes after submersion but is reduced to between 2 and 3 volumes per cent by the end of 6 minutes and is almost completely exhausted within 7 or 8 minutes They observed that the carbon dioxide content of whole blood rises rapidly during the first few minutes of submersion but by the end of 4 minutes drops below the pre-experimental level Subsequently it undergoes a slowly progressive decline A similar antemortem drop was observed in the total carbon dioxide of the blood of animals killed by tracheal compression

If depletion of oxygen and retention of carbon dioxide are the primary effects of obstructive asphyxia a redistribution of electrolytes between cells and plasma and a lowering of the pH of the blood may be characterized as secondary attributes Although these secondary changes might be of diagnostic value if recognized during life their presence in postmortem blood can have no meaning beyond the fact that circulation has ceased, and that death has occurred Other and, from a diagnostic standpoint, equally unimportant asphyxial changes in the composition of the blood result from hemoconcentration (Collip, Peters and Van Slyke, Inouye and Uchimura)

Despite the fact that the absence of oxygen in a postmortem sample of blood has no diagnostic significance its presence may provide conclusive evidence that death did not result from asphyxia Goggio found that the oxygen content of arterial blood is regularly reduced to a trace after asphyxial death whereas in animals dead of sudden cardiac arrest (electrical shock) many hours elapse before the residual oxygen of arterial blood is reduced to a level consistent with death from asphyxia

That asphyxia commonly results in a marked rise in the glucose concentration of the blood has been known since the early investigations of Claude Bernard Although various stimuli may cause a sudden outpouring of adrenalin resulting in hepatic glycogenolysis and hyperglycemia, Hill has recently stressed the fact that a high glucose concentration in an appropriate sample of blood may provide evidence of confirmatory value in the recognition of acute asphyxial death It should be remembered, however, that the finding of a high glucose concentration in a postmortem sample of blood from the right side of the heart does not necessarily indicate an antemortem state of hyperglycemia, nor does the absence of glucose from a postmortem sample of blood indicate hypoglycemia during life A sharp, evanescent elevation of the glucose in the inferior vena cava and right side of the heart as a result of hepatic glycogenolysis is a normal postmortem phenomenon If a postmortem sample of blood is to be taken for glucose determination it should be from the left heart and it should be taken as soon after death as possible to avoid the artefact of postmortem glycolysis

*Chemical changes due to the exchange of water and electrolytes between inhaled fluid and the blood in the pulmonary capillaries* More than a century ago Mayer demonstrated that the lungs are capable of absorbing water and certain water solutes That the absorbed material may alter the composition of the blood was indicated by the observation that within two minutes after the fluid had reached the pulmonary alveoli certain chemicals present in it could be demonstrated in the chamber of the left ventricle

Peiper reported that a dog could inhale as much as 250 cc of inert fluid in the course of an hour without suffering from dyspnea Sehrwald described an experiment in which 775 grams of fluid were introduced into the lungs of a 16 lb dog over a period of 2 hours without causing undue distress Clinical evidence indicated that the fluid was absorbed by the end of 5 days Colin introduced 25 liters of water into the trachea of a horse over a period of 6 hours without significant interference with pulmonary ventilation The horse was protected against hydremia, however, by the withdrawal of 6 kilograms of blood during the course of the experiment Karpovich in an experimental study with rats tied the esophagus of his animals before submerging them and observed that the amount of fluid retained in their bodies after drowning often exceeded the weight of the lungs by more than 100 per cent

The question of whether or not submersion of a body after death may result in changes in the composition of the blood similar to those caused by drowning has been studied by various investigators Small amounts of water may enter the trachea and bronchi of the bodies of men (Müller) and of animals (Wachholz) that have been submerged after death from causes other than drowning It was found, however, that water does not enter the small air passages or pulmonary alveoli in such circumstances That diffusion between the water that has entered the air passages after death and the blood in the pulmonary capillaries is not sufficient to produce significant changes in heart's blood was shown by Jetter and Moritz

Various investigators have added chemical tracers to the fluid in which ani

mals were drowned in order to demonstrate absorption from the lungs Doehne in 1857, Falk in 1869, and Sehrwald in 1886 drowned animals in fluid containing potassium ferrocyanide and subsequently demonstrated the presence of that chemical in the blood stream More recently Lochte and Danziger added potassium iodide and Lochte added calcium oxide to the fluid in which animals were drowned Winternitz demonstrated the absorptive capacity of the respiratory membrane by adding phenolsulphonphthalein to water and observed that dye is absorbed as rapidly from the alveoli as it is when injected intramuscularly

Brouardel conducted the first experimental investigation of the mechanism of death in drowning by what might be characterized as modern physiological methods Stimulated in part by his own observations on the absorption of inhaled water and in part by his appreciation of the unreliability of postmortem pathological changes in making a diagnosis of death by drowning, he undertook to investigate the diagnostic significance of disproportionate intracardiac hemodilution From observations made in collaboration first with Vibert and later with Loye he concluded that a comparison of the hemoglobin and erythrocyte content of right and left heart's blood constituted a practical method of recognizing death by drowning By both procedures it was observed that disproportionate dilution of left heart's blood occurred after drowning in fresh water Brouardel's observations were subsequently confirmed by Paltauf

When consideration is given to the nature of the terminal circulatory failure in death by drowning it is not surprising that at autopsy the blood in the left side of the heart may differ from that in the right After consciousness has been lost and the glottic spasm relaxed, enough water is usually inhaled to change the composition of the blood passing through the pulmonary capillaries It can be assumed that the nature and degree of change will depend on the differences that exist between the osmotic pressure of the intra-alveolar fluid and that of capillary blood and on the length of time that circulation is maintained during and after the inhalation of water If heart failure in drowning were sudden so as to cause an abrupt and complete cessation of circulation the finding of significant postmortem differences in the composition of the blood in the two sides of the heart would be unlikely It has been observed however that in experimental animals there is electrocardiographic evidence of feeble contractions for as long as 30 to 40 minutes after all respiratory efforts have ceased and after the pulse has become imperceptible (Lougheed, Janes and Hall) During this period of agonal circulatory failure it is to be expected that blood which has been altered in passing through the pulmonary capillaries will be gradually pooled in the dilated vessels of the systemic circulation so that each feeble ventricular discharge will increase the change in the left heart's blood without contributing correspondingly to the change in the right side Thus, when drowning has occurred in fresh water it would be reasonable to expect to find disproportionate dilution of left heart's blood By the same reasoning it might be expected that when drowning occurs in an aqueous medium containing a sufficient concentration of electrolytes to raise its osmotic pressure above that of blood, a disproportionate increase in these electrolytes will be found in a postmortem

sample of left ventricular blood. Sea water which usually has a mineral concentration more than three times greater than that of the blood represents such a medium.

Presumptive evidence of disproportionate dilution might be obtained from a comparison of the hemoglobin, the iron, or the number of red cells in samples taken from the right and left sides of the heart. It is obvious, however, that the significance of such observations will depend on how truly the postmortem samples represent the plasma-cell ratio of the blood at the moment of death. Postmortem settling of erythrocytes or movement of plasma can and does account for uncontrollable factors of error.

Two other types of criteria for judging the occurrence of agonal diffusion may be considered. One is by the recognition of changes in the molecular concentration of blood samples by such physical characteristics as specific gravity, freezing point, or electrical conductivity. The other is the identification by chemical means of alterations in the electrolyte concentration of the blood. Thus, after drowning in fresh water a reduction might be expected in the specific gravity, the $\Delta$ of the freezing point and the electrical conductivity of the left heart's blood, whereas after drowning in sea water the reverse should be true. After drowning in fresh water electrolytes in the left ventricle might be expected to be disproportionately diminished whereas an increase would be expected after drowning in sea water.

Carrara was among the first to apply physical methods to investigate the effects of drowning on the molecular concentration of heart's blood. As a result of determinations of specific gravity, freezing point and electrical conductivity he concluded that left ventricular blood is disproportionately diluted after drowning in fresh water and disproportionately concentrated after drowning in sea water. He also observed that postmortem alterations in blood independently of drowning may account for equally great changes and concluded, perhaps prematurely, that it is impossible in non-experimental conditions to make a categorical statement regarding the diagnostic significance of changes found in postmortem samples.

During the succeeding 25 years the diagnostic significance of hemo-dilution and -concentration as disclosed by a variety of physical tests was the subject of conflicting opinions. Placzek recommended the diagnostic value of determining the specific gravity of right and left heart's blood. Revenstorf proposed cryoscopic examination and although he recognized the fact that postmortem changes, independent of drowning, often result in pronounced and unpredictable alteration in the density of the blood, concluded that the procedure was frequently of diagnostic value in borderline cases. He called attention to the fact that postmortem depression of the freezing point normally occurs more rapidly in blood than it does in spinal fluid, whereas, after death by drowning in fresh water, the freezing point of left ventricular blood was likely to be higher than that of the spinal fluid. Balthazard concluded that cryoscopic examination was superior to determining either the hemoglobin or erythrocyte content of the blood, whereas Wachholz and Horoszkiewicz doubted the diagnostic reliability of either

procedure. Canuto on a basis of uncontrolled data concluded that recognition of disproportionate changes in the refractive index of right and left heart's blood provides a basis for establishing a postmortem diagnosis of death by drowning in man. Yamakami, and later Inouye and Uchimura, confirmed the already established fact that changes in the freezing point and refractive index occur in the blood of experimentally drowned animals. Schwarzacher, in a reinvestigation of the significance of alterations in electrical conductivity of the blood of persons dead of drowning and of persons dead of causes other than drowning, concluded that the postmortem artefact rendered such tests of little or no practical value.

Although Stockis was first to report differences in the chloride content of right and left heart's blood after death by drowning he was not particularly impressed with their diagnostic significance and not until 1921 when Gettler reported a relatively large series of determinations on human subjects was their potential diagnostic significance appreciated. On a basis of chloride determinations of right and left heart's blood of 22 individuals dead of causes other than drowning, 19 deaths by drowning in salt water and 3 deaths by drowning in fresh water, Gettler concluded that a difference of 25 mgm. NaCl establishes death by drowning. He called attention to the fact that a significant difference might fail to develop if death resulted from the shock of immersion rather than drowning or if there were a patent foramen ovale. According to Gettler a high chloride value in the left ventricle indicates death by drowning in salt water and low chloride value signifies drowning in fresh water. It was observed that the longer the interval between inhalation of water and death the greater the differences were likely to be.

Since the publication of Gettler's observations the significance of the chloride content of the blood in relation to death by drowning has been the subject of numerous investigations and considerable diversity of opinion. In order to evaluate the published data in their entirety all chloride values have been converted to milli-equivalents per liter and arranged in tabular form. Some authors described analytical procedures and others did not. From the available descriptions it is apparent that all methods were not of equal sensitivity and certain differences in the data are probably attributable to this fact.

*Blood chlorides (rabbits) after death from causes other than drowning.* It is obvious that knowledge of the nature and extent to which the blood chlorides may be altered incident to death from causes other than inhalation of water is pre-requisite to the evaluation of changes regarded as pathognomonic of drowning. The chloride concentrations of whole blood or serum of 15 rabbits taken before and immediately after death by strangulation are shown in table 1. Antemortem samples were from the ear vein and postmortem samples from the right side of the heart. In every instance the postmortem was higher than the antemortem value, the increases ranging from 3 to 9 mEq/L in whole blood and from 2 to 8 in serum. So far as can be judged from the limited number of observations the increases in serum and whole blood are of approximately the same order of magnitude. In the 4 animals reported by Inouye and Uchimura it was observed that the refractive index of the serum was increased and that its freezing point was reduced.

In another series of control experiments (see table 2) the chloride concentration of postmortem samples of right and left heart's blood taken immediately after death by strangulation was determined. It appears that the increased concentration observed after death from mechanical asphyxia (strangulation) does not affect the blood in one side of the heart more than it does in the other. The recorded differences are not regarded to be significant.

TABLE 1

*Chloride content of rabbits' blood* before and immediately after death by tracheal compression (milli-equivalents per liter)*

| ANTE MORTEM | POST MORTEM | DIFFERENCE, PM–AM | REPORTED BY | ANTE MORTEM | POST MORTEM | DIFFERENCE, PM–AM | REPORTED BY |
|---|---|---|---|---|---|---|---|
| 91 | 99 | 8 | Yamakami | 87 | 96 | 9 | Yamakami |
| 85 | 90 | 5 | Yamakami | 87 | 94 | 7 | Yamakami |
| 85 | 95 | 10 | Yamakami | 83* | 85* | 2 | Inouye and Uchimura |
| 89 | 101 | 12 | Yamakami | 100* | 106* | 6 | Inouye and Uchimura |
| 90 | 93 | 3 | Yamakami | 97* | 105* | 8 | Inouye and Uchimura |
| 87 | 91 | 4 | Yamakami | 92* | 97* | 5 | Inouye and Uchimura |
| 88 | 91 | 3 | Yamakami | | | | |

* Values designated by * represent serum. All other values are for whole blood.

TABLE 2

*Chloride content of right and left heart's blood* of rabbits immediately after death by tracheal compression (milli-equivalents per liter)*

| RIGHT HEART | LEFT HEART | DIFFERENCE, LH–RH | REPORTED BY | RIGHT HEART | LEFT HEART | DIFFERENCE, LH–RH | REPORTED BY |
|---|---|---|---|---|---|---|---|
| 97 | 97 | 0 | Yamakami | 96 | 94 | −2 | Yamakami |
| 93 | 92 | −1 | Yamakami | 93 | 93 | 0 | Yamakami |
| 85 | 85 | 0 | Yamakami | 98 | 97 | −1 | Yamakami |
| 91 | 91 | 0 | Yamakami | 98 | 98 | 0 | Yamakami |
| 101 | 101 | 0 | Yamakami | 99 | 100 | 1 | Yamakami |
| 93 | 93 | 0 | Yamakami | 94 | 95 | 1 | Yamakami |
| 91 | 92 | 1 | Yamakami | 106* | 106* | 0 | Inouye and Uchimura |
| 91 | 91 | 0 | Yamakami | 106* | 107* | 1 | Inouye and Uchimura |
| 91 | 90 | −1 | Yamakami | 111* | 112* | 1 | Inouye and Uchimura |
| 93 | 91 | −2 | Yamakami | | | | |

* Values designated * represent serum. All other values are for whole blood.

*Blood chlorides (rabbits) after death by drowning in fresh water.* The chloride concentrations of right and left heart's blood of 20 rabbits taken immediately after death by drowning in fresh water are shown in table 3. It is apparent that in every animal there has been a significant and disproportionate reduction in the chloride content of the blood in the left side of the heart which persisted after death. The mean value of the differences between the 2 sides of the heart was 17 mEq/L with a minimum of 6 and a maximum of 27. Although the data shown in table 3 do not indicate whether or not the right heart's blood was also diluted,

the pre-experimental blood chloride values for the 2 animals reported by Inouye and Uchimura did not indicate that such was the case The pre-experimental serum chloride values for venous blood from these 2 animals were respectively 101 and 107 mEq/L

*Blood chlorides (rabbits) after drowning in salt water* The chloride concentrations of right and left heart's blood of 15 rabbits observed immediately after death

TABLE 3

*Chloride content of right and left heart's blood* of rabbits immediately after death by drowning in fresh water (milli-equivalents per liter)*

| RIGHT HEART | LEFT HEART | DIFFERENCE, LH-RH | REPORTED BY | RIGHT HEART | LEFT HEART | DIFFERENCE, LH-RH | REPORTED BY |
|---|---|---|---|---|---|---|---|
| 104* | 80* | −24* | Inouye and Uchimura | 85 | 71 | −14 | Yamakami |
| 105* | 78* | −27* | Inouye and Uchimura | 73 | 55 | −18 | Yamakami |
| 79 | 71 | −8 | Yamakami | 82 | 67 | −15 | Yamakami |
| 80 | 64 | −16 | Yamakami | 83 | 60 | −23 | Yamakami |
| 85 | 63 | −22 | Yamakami | 80 | 58 | −22 | Yamakami |
| 94 | 88 | −6 | Yamakami | 74 | 62 | −12 | Yamakami |
| 79 | 66 | −13 | Yamakami | 80 | 70 | −10 | Yamakami |
| 88 | 62 | −26 | Yamakami | 85 | 69 | −16 | Yamakami |
| 82 | 65 | −17 | Yamakami | 88 | 72 | −16 | Yamakami |

* Values designated * represent serum All other values are for whole blood

TABLE 4

*Chloride content of right and left heart's blood* of rabbits immediately after drowning in salt water (milli-equivalents per liter)*

| RIGHT HEART | LEFT HEART | DIFFERENCE LH-RH | REPORTED BY | RIGHT HEART | LEFT HEART | DIFFERENCE LH-RH | REPORTED BY |
|---|---|---|---|---|---|---|---|
| 135* | 148* | 13 | Inouye and Uchimura | 112 | 151 | 39 | Yamakami |
| 116* | 123* | 7 | Inouye and Uchimura | 102 | 114 | 12 | Yamakami |
| 120* | 137* | 17 | Inouye and Uchimura | 117 | 139 | 22 | Yamakami |
| 129* | 154* | 25 | Inouye and Uchimura | 116 | 136 | 20 | Yamakami |
| 106 | 131 | 25 | Yamakami | 125 | 141 | 16 | Yamakami |
| 137 | 166 | 29 | Yamakami | 117 | 132 | 15 | Yamakami |
| 134 | 160 | 25 | Yamakami | 120 | 146 | 26 | Yamakami |
| 127 | 169 | 42 | Yamakami | | | | |

* Values designated * represent serum All other values are for whole blood

from drowning in salt water are shown in table 4 In every instance the concentration was greater in the left than in the right side of the heart The mean value of the differences for whole blood (11 pairs of samples) was 25 mEq/L and for serum (4 pairs of samples) was 16

It is apparent not only that the chloride concentrations in the left heart were higher than those in the right side of the heart but also that the chloride concentrations on both sides are higher than would be expected in animals dead of causes

other than drowning The difference between chambers ranged from 7 and 42 mEq/L In addition to the data shown in table 4 antemortem chloride values were available for the 4 rabbits reported by Inouye and Uchimura In each of these the chloride content of the postmortem samples of venous blood (right heart) was significantly higher than that of the corresponding antemortem samples The differences between the antemortem and postmortem samples in the 4 animals were respectively 13, 7, 17 and 25 mEq/L

TABLE 5

*Chloride content of blood serum taken from dogs before and at varying intervals after death by tracheal compression (Jetter and Moritz) (milli-equivalents per liter)*

| ANTEMORTEM | POSTMORTEM INTERVAL | RIGHT HEART | LEFT HEART | DIFF BETWEEN A.M AND P.M SAMPLES OF VENOUS BLOOD P.M.-A.M | POSTMORTEM DIFF BETWEEN RH AND LH, LH-RH |
|---|---|---|---|---|---|
| | *hrs.* | | | | |
| 115 | ½ | 117 | 121 | 2 | 4 |
| | 6 | 114 | 119 | −1 | 5 |
| | 24 | 78 | 78 | −37 | 0 |
| | 48 | 74 | 72 | −41 | −2 |
| 116 | ½ | 117 | 121 | 1 | 4 |
| | 12 | 88 | 100 | −28 | 12 |
| | 24 | 78 | 86 | −38 | 8 |
| 114 | 6 | 114 | 116 | 0 | 2 |
| | 12 | 107 | | −7 | |
| | 24 | 84 | 95 | −30 | 11 |
| | 48 | 65 | | −49 | |
| | 72 | 60 | | −54 | |
| 134 | 24 | 72 | 78 | −62 | 6 |
| | 48 | 71 | 68 | −63 | −3 |
| | 72 | 65 | 64 | −69 | −1 |
| 106 | 24 | 79 | 89 | −27 | 10 |
| | 72 | 77 | 77 | −29 | 0 |

From determinations of chlorides, freezing point, and refractive index of rabbit serum before and after death Inouye and Uchimura concluded that whereas the reduction of chlorides incident to drowning in fresh water is consistent with simple dilution by diffusion, the chloride concentration after salt water drowning could not be accounted for by diffusion alone

*Blood chlorides (dogs) after death from causes other than drowning* The chloride content of blood serum taken from dogs before and at different intervals after death by tracheal compression is shown in table 5 In all animals there was a progressive decline in the chloride concentration of both right and left heart's blood as the interval between death and sampling lengthened Significant

diminution first became apparent between 6 and 12 hours after death The percentile loss at the end of 24 hours from the right heart of the 5 dogs shown in table 5 was respectively 32, 33, 26, 46 and 25 The corresponding percentile chloride decreases in the left heart were 32, 26, 17, 42 and 16 Thus it appears that in dogs postmortem reduction of serum chlorides tends to take place more rapidly in the right than in the left side of the heart In the case of the former the antemortem values were reduced by between a quarter and a half during the first 24 hours after death As a result of variations in the rate of chloride loss from the right and left sides of the heart of the same animal differences as great as 12 mEq/L may be encountered during the first 12 hours after death

That the postmortem reduction in chlorides occurred more rapidly from serum than from whole blood was apparent from unpublished experimental data of Jetter and Moritz It was furthermore observed that although the environmental temperature had little or no effect on the rate of serum chloride loss during the first day after death the rapidity of chloride loss during the second and succeeding days was enhanced by putrefaction

Several factors undoubtedly contribute to the change that occurs in the chloride content of blood after death When freshly drawn whole blood is stored in a test tube it will be found that there is a progressive shift of chlorides from plasma to cells Imbibition by erythrocytes may account for a drop in plasma chlorides by as much as 10 per cent in 48 hours (Jetter and Moritz) It is apparent, however, that a reduction in the total chlorides of whole blood can only be accounted for by their diffusion out of the vessels and into the surrounding tissues In life the antemortem chloride content of the tissues is much lower than that of the plasma Thus the chloride content of heart muscle is approximately 50 per cent and that of skeletal muscle approximately 20 per cent of that of the plasma On the other hand the magnesium content of cellular fluid is many times greater than that of plasma (Shohl) During life the maintenance of the chemical individuality of blood and tissues depends in part upon the ability of the cell membranes to regulate the diffusion of substances through them and in part upon the continuance of metabolic activity within cells whereby the integrity of their various organic constituents is preserved After death not only do cell membranes become more permeable but the organic constituents of cytoplasm deteriorate so as to predispose to a free exchange of diffusible substances between the intra- and extracellular fluids in the direction of chemical homogeneity Thus regardless of the cause of death the postmortem migration of chlorides out of the plasma and into cells and of magnesium out of the cells and into the plasma is to be expected Data concerning the alterations that occur in the magnesium content of the blood incident to postmortem change as well as to drowning are presented in a later section of this review

*Blood chlorides (dogs) after drowning in fresh water* The blood chloride levels of 7 dogs drowned in fresh water are shown in table 6 Only in the first 2 were samples obtained from both the right and left sides of the heart In the first dog the disproportionate reduction of chlorides in the left ventricle during the first 24 hours after death was greater than had been observed in any animal in the

control series It is to be noted, however, that the greatest difference between the chloride levels in the two sides of the heart of this animal existed immediately after death and that the differences became progressively smaller as the postmortem interval lengthened Although the chloride reduction was greater in the left, the right heart values were also depressed Within 15 minutes after death the chlorides in the right heart were 52 mEq/L lower than in the antemortem sample In no control animal was a reduction of this magnitude observed earlier than 24 hours after death

In the second dog there was bilateral reduction in the chloride levels but the differences between the right and left sides of the heart did not exceed those encountered in control animals The third dog also revealed evidence of bilat

TABLE 6

*Chloride content of blood* of dogs taken before and at varying intervals after death by drowning in fresh water (milli-equivalents per liter)*

| ANTE MORTEM | POSTMORTEM INTERVAL | RIGHT HEART | LEFT HEART | POSTMORTEM DIFF BETWEEN RH AND LH LH–RH | DIFF BETWEEN P.M AND A.M. SAMPLES OF VENOUS BLOOD P.M–A.M | REPORTED BY |
|---|---|---|---|---|---|---|
| 128 | ¼ hr | 76 | 38 | −38 | −52 | Jetter and Moritz |
| | 12 hrs | 72 | 41 | −31 | −56 | |
| | 24 hrs | 64 | 42 | −22 | −64 | |
| | 3 days | 40 | 40 | −9 | −79 | |
| 112 | ¼ hr | 96 | 99 | 3 | −16 | Jetter and Moritz |
| | 6 hrs | 83 | 86 | 3 | −29 | |
| | 24 hrs | 76 | 68 | −8 | −36 | |
| 108 | 0 hr | 71 | | | −37 | Guislain |
| 102 | 24 hrs | 55 | | | −47 | Guislain |
| 104 | 3 days | 44 | | | −60 | Guislain |
| 108 | 7 days | 42 | | | −66 | Guislain |
| 106 | 12 days | 39 | | | −67 | Guislain |

* Values designated * represent whole blood All other values are for serum

eral dilution of heart's blood but the low chloride values of the remaining animals could as well be attributed to postmortem change as to drowning

Although the available experimental data concerning blood chloride concentration in dogs after drowning in fresh water are not sufficiently numerous to draw conclusions regarding the extent to, or the frequency with, which drowning in fresh water may result in pathognomonic change, it is clear that a sufficient degree of reduction may occur in the left side of the heart to be of diagnostic significance It is also clear that unless the blood is obtained soon after death (within 24 hrs) differences that might otherwise be of diagnostic value are likely to be masked by postmortem diffusion

*Blood chlorides (dogs) after drowning in salt water* The chloride concentrations of the blood of 13 dogs before and after death by drowning in salt water are shown in table 7 In all instances in which the samples were collected within the first 24 hours after death the serum chlorides were not only disproportionately high in

the left side but the differences between right and left sides were greater than had been observed in any animals of the control series In the first 3 dogs the elevation was bilateral whereas in the last 10 chlorides were increased only in the left side

In animals that were sampled repeatedly during the postmortem interval (1, 2, 3) it was observed that the disparity between the chloride concentrations

TABLE 7

*Chloride content of blood of dogs taken before and at varying intervals after death by drowning in salt water (milli-equivalents per liter)*

| ANTEMORTEM | POSTMORTEM INTERVAL | RIGHT HEART | LEFT HEART | POSTMORTEM DIFF BETWEEN RH AND LH, LH–RH | DIFF BETWEEN P.M AND A.M SAMPLES OF VENOUS BLOOD, P.M –A.M | REPORTED BY |
|---|---|---|---|---|---|---|
| *serum* | *hrs* | *serum* | *serum* | | | |
| 123 | ½ | 136 | 151 | 15 | 15 | Jetter and Moritz |
| | 6 | 114 | 161 | 47 | −9 | |
| | 12 | 112 | 169 | 57 | −11 | |
| | 48 | 95 | 96 | 1 | −28 | |
| 123 | 12 | 139 | 152 | 13 | 16 | Jetter and Moritz |
| | 24 | 110 | 135 | 25 | −13 | |
| | 48 | 107 | 119 | 12 | −16 | |
| | 72 | 108 | 114 | 6 | −15 | |
| 124 | 12 | 132 | 154 | 22 | 6 | Jetter and Moritz |
| | 24 | 95 | 123 | 28 | −29 | |
| | 48 | 93 | 104 | 11 | −31 | |
| | 72 | 91 | 96 | 5 | −33 | |
| *whole blood* | | *whole blood* | *whole blood* | | | |
| 104 | 0 | 101 | 141 | 40 | −3 | Tarsitano |
| 103 | 0 | 99 | 143 | 44 | −4 | Tarsitano |
| 106 | 0 | 109 | 131 | 22 | 3 | Tarsitano |
| 101 | 0 | 99 | 137 | 38 | −2 | Tarsitano |
| 107 | 0 | 103 | 142 | 39 | −4 | Tarsitano |
| 103 | 0 | 102 | 144 | 42 | −1 | Tarsitano |
| 106 | 0 | 103 | 143 | 40 | −1 | Tarsitano |
| 108 | 0 | 108 | 130 | 22 | 0 | Tarsitano |
| 102 | 0 | 102 | 141 | 39 | 0 | Tarsitano |
| 98 | 0 | 98 | 137 | 39 | 0 | Tarsitano |

in the right and left sides of the heart increased during the first 24 hours and decreased thereafter Despite the fact that the disproportion in the chloride concentration in the two sides of the heart became less pronounced after 24 hours, its mean concentration remained higher than that of the control animals

*Blood chlorides (man) after death from causes other than drowning* Chloride values for right and left heart's blood of 46 persons dead of causes other than

drowning are given in table 8. In 21 the interval between death and sampling was stated. The other 25 were reported without specifying the time between death and autopsy. Although a variety of causes, both natural and violent, were represented, there was no consistent relationship between the cause of death and the rate of postmortem change in the chloride concentration.

If 99 mEq/L be regarded as the antemortem lower limit of normal for chlorides in serum, and 77 for whole blood (Gram, Peters and Van Slyke), it is apparent that a reduction in blood chlorides is a common if not a constant postmortem phenomenon. In 90 per cent of the cases in which postmortem samples of serum were analyzed the chloride level in one or both sides of the heart was below the lower antemortem limit of normal whereas in less than 25 per cent of the cases in which whole blood was analyzed did the concentration fall below the antemortem range.

That neither the intravascular shift nor the extravascular diffusion necessarily progress at the same rate on the two sides of the heart is indicated by the frequency with which dissimilar right and left heart values were encountered. Thus it may be seen in table 8 that the left heart serum values ranged from 7 milli-equivalents lower to 14 milli-equivalents higher than their corresponding right heart values. The chloride values for whole blood from the left heart ranged from 13 milli-equivalents lower to 8 milli-equivalents higher than their corresponding right heart values. A disparity as great as 8 mEq/L may develop within the first 5 hours after death from causes other than drowning. If diagnostic significance is to be attached to a difference in the chloride content of right and left heart's blood in cases of suspected drowning the difference should exceed those observed after comparable postmortem intervals in persons dead of causes other than drowning.

*Blood chlorides (man) after drowning in fresh water.* The results of chloride determinations on blood samples obtained at postmortem examination from the bodies of 34 persons presumably dead of drowning in fresh water are shown in table 9. In 14 information was available concerning the length of the interval between death and sampling. In 20 the analytical results were reported without specific reference to the length of the postmortem interval.

In 26 of the 34 cases blood was taken from both sides of the heart. In only one of these was the interval between drowning and sampling stated to be less than 12 hours. In this case autopsy was performed approximately 8 hours after death and the analysis disclosed a greater relative reduction in left heart chlorides than had been observed in any of the control cases. In the other 13 cases of right and left heart sampling in which the length of the postmortem interval was stated, the differences did not exceed those encountered in the control series. It may or may not be significant that in all 13 the left heart chloride levels were lower than those in the right.

It is difficult to assess the significance of blood chloride values in the absence of information concerning the interval between death and sampling. Soutter stated that in none of his cases was the interval between death and autopsy greater than 6 days. In one of Soutter's cases of fresh water drowning, blood

## TABLE 8

*Postmortem changes in the chloride concentrations of human blood*

Deaths from causes other than drowning (milli-equivalents per liter)

| SERIES I—POSTMORTEM INTERVAL STATED | | | | | | | | SERIES II—POSTMORTEM INTERVAL NOT SPECIFIED | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Postmortem interval | Whole Blood | | | Serum | | | Reported by | Postmortem interval | Whole Blood | | | Reported by |
| | RH | LH | L-R | RH | LH | L-R | | | RH | LH | L-R | |
| *hrs* | | | | | | | | | | | | |
| 5 | 89 | 88 | −1 | 100 | 108 | 8 | Moritz and McLean | ? | 83 | 83 | 0 | Gettler |
| 12 | | | | 100 | 94 | −6 | Moritz and McLean | ? | 86 | 85 | −1 | Gettler |
| 12 | 90 | 89 | −1 | 92 | 101 | 9 | Soutter | ? | 84 | 84 | 0 | Gettler |
| 16 | | | | 78 | 77 | −1 | Moritz and McLean | ? | 84 | 84 | 0 | Gettler |
| 18 | 88 | 96 | 8 | 96 | 110 | 14 | Moritz and McLean | ? | 77 | 76 | −1 | Gettler |
| 18 | 78 | 65 | −13 | 90 | 90 | 0 | Moritz and McLean | ? | 81 | 80 | −1 | Gettler |
| 18 | 81 | 85 | 4 | 96 | 89 | −7 | Moritz and McLean | ? | 95 | 95 | 0 | Gettler |
| 18 | | | | 80 | 90 | 10 | Soutter | ? | 87 | 87 | 0 | Gettler |
| 18 | 90 | 88 | −2 | 95 | 90 | −5 | Soutter | ? | 84 | 84 | 0 | Gettler |
| 20 | 59 | 59 | 0 | | 59 | | Moritz and McLean | ? | 82 | 81 | −1 | Gettler |
| 22 | | | | 107 | 104 | −3 | Moritz and McLean | ? | 120 | 119 | −1 | Gettler |
| 24 | 87 | 84 | −3 | 92 | 95 | 3 | Moritz and McLean | ? | 85 | 84 | −1 | Gettler |
| 24 | 86 | 86 | 0 | 91 | 90 | −1 | Moritz and McLean | ? | 82 | 82 | 0 | Gettler |
| 24 | | | | 69 | 77 | 8 | Soutter | ? | 85 | 84 | −1 | Gettler |
| 24 | 59 | 59 | 0 | 62 | 62 | 10 | Moritz and McLean | ? | 90 | 89 | −1 | Gettler |
| 25 | 72 | 70 | −2 | | | | Soutter | ? | 77 | 78 | 1 | Gettler |
| 25 | 77 | 82 | 5 | | | | Soutter | ? | 58 | 58 | 0 | Gettler |
| 30 | 84 | 84 | 0 | 84 | 86 | 2 | Soutter | ? | 88 | 88 | 0 | Gettler |
| 30 | 86 | 84 | −2 | 90 | 86 | −4 | Soutter | ? | 121 | 120 | −1 | Gettler |
| 36 | 72 | 76 | 4 | | | | Soutter | ? | 101 | 100 | −1 | Gettler |
| 36 | | | | 56 | 51 | −5 | Moritz and McLean | ? | 92 | 92 | 0 | Gettler |
| | | | | | | | | ? | 90 | 88 | −2 | Gettler |
| | | | | | | | | ? | 63 | 62 | −1 | Palmer and Doherty |
| | | | | | | | | ? | 88 | 75 | −13 | Palmer and Doherty |
| | | | | | | | | ? | 68 | 63 | −5 | Palmer and Doherty |

## TABLE 9

*Chloride concentrations of blood of human subjects probably dead of drowning in fresh water (milli-equivalents per liter)*

| POSTMORTEM INTERVAL | WHOLE BLOOD | | | SERUM | | | REPORTED BY | POSTMORTEM INTERVAL | WHOLE BLOOD | | | SERUM | | | REPORTED BY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RH | LH | L-R | RH | LH | L-R | | | RH | LH | L-R | RH | LH | L-R | |
| *days* | | | | | | | | | | | | | | | |
| ⅓ | | | | 99 | 6 | −23 | Moritz and McLean | <6 days | 80 | 80 | 0 | | | | Soutter |
| ½ | 92 | 89 | −3 | | | | Stockis | <6 days | 58 | 80 | 22 | | | | Soutter |
| 1 | | | | 55 | 56 | 1 | Moritz and McLean | <6 days | 66 | 82 | 16 | | | | Soutter |
| 1 | 74 | | | | | | Müller | <6 days | | | | 95 | 89 | −6 | Soutter |
| 2 | 74 | 68 | −6 | 88 | 82 | −6 | Moritz and McLean | <6 days | | | | 78 | 80 | 2 | Soutter |
| 2 | 114 | 113 | −1 | | | | Stockis | <6 days | | | | 72 | 80 | 8 | Soutter |
| 2–3 | 52 | | | | | | Müller | <6 days | | | | 86 | 82 | −4 | Soutter |
| 3 | 65 | 58 | −7 | | | | Moritz and McLean | <6 days | | | | 88 | 88 | 0 | Soutter |
| 4–5 | 54 | | | | | | Müller | <6 days | | | | 85 | 88 | 3 | Soutter |
| 4–5 | 80 | | | | | | Müller | <6 days | 70 | 78 | 8 | | | | Soutter |
| 5 | 54 | | | | | | Müller | <6 days | 67 | 64 | −3 | | | | Soutter |
| 5–6 | 79 | | | | | | Leclerc | Unspecified | 107 | 85 | −22 | | | | Gettler |
| 8–10 | 60 | | | | | | Müller | Unspecified | 74 | 63 | −11 | | | | Gettler |
| 30 | 54 | | | | | | Müller | Unspecified | 92 | 85 | −7 | | | | Gettler |
| <6 | 58 | 58 | 0 | | | | Soutter | Unspecified | ? | ? | −1 | | | | Palmer |
| <6 | 70 | 68 | −2 | | | | Soutter | Unspecified | ? | ? | −3 | | | | Palmer |
| <6 | 76 | 71 | −5 | | | | Soutter | Unspecified | ? | ? | −3 | | | | Palmer |

from the left heart contained 22 milli-equivalents more chlorides per liter than did the blood from the right side. Not only did this difference exceed any of those encountered in the control group but it was the reverse of what one would be led to expect from the results of animal experimentation. In general Soutter's findings in deaths presumably due to fresh water drowning are at variance with those of other observers.

Until chloride determinations made within 12 hours after death are available from a larger series of human cases of fresh water drowning final judgment regarding their value must be held in abeyance. Although there is adequate evidence from experimental animals (rabbits) of the diagnostic value of blood chloride determinations immediately after death from drowning in fresh water, it is clear from data derived from dogs and man that values obtained 12 or more hours after death are frequently of little or no diagnostic value.

In a human body recovered from fresh water a disproportionate depression of the chlorides in the left side of the heart of 17 milli-equivalents or more per liter should probably be regarded as presumptive evidence of drowning. Failure to find a significant postmortem difference in the chloride content on the two sides of the heart of such a body should not be regarded as evidence that death resulted from causes other than drowning.

In 8 of the 34 cases included in table 9 samples were taken from only the right side of the heart. Although the chloride values for these samples are lower than would be expected during life they are not significantly lower than those frequently observed in persons dead of causes other than drowning.

*Blood chlorides (man) after drowning in sea water.* Chloride determinations on samples of right and left heart's blood in 32 persons thought to have died of drowning in sea water are included in table 10. If 90 mEq/L of chloride represents the upper antemortem limit of normal for whole blood and 108 for serum (Gram, Peters and VanSlyke) abnormally high chloride values were present in over 80 per cent of persons dead of drowning in salt water. Another significant feature of the data shown in table 10 is that in 19 of the 32 cases of drowning in salt water the chloride level was not only higher in the left heart but the differences between the right and the left heart were greater than had been observed in any of the control series. It appears, therefore, that postmortem change is less likely to mask or simulate the effects of drowning in salt water than those of drowning in fresh water. It may be inferred from the published data that a preponderance of chlorides in blood from the left side of the heart of 17 mEq or more per liter constitutes presumptive evidence of drowning in salt water.

*Postmortem chloride content of pleural, pericardial, and peritoneal blood.* In an effort to determine whether drowning leads to disproportionate dilution of fluids other than blood Guislain compared the postmortem chloride values of blood and pleural fluid of 5 dogs killed by cerebral trauma with those of 5 dogs that had been drowned in fresh water. Both the blood and the pleural fluid of the drowned animals showed lower chloride values than were observed in the controls and the chloride reductions in the pleural fluid were of the same order of magnitude as those of the blood.

Analyses of pleural, pericardial, and peritoneal fluids of human subjects dead of causes other than drowning are shown in table 11 Insofar as it is possible to interpret these data it appears that postmortem loss of chloride occurs most rapidly from peritoneal fluid, less rapidly from the blood and pleural fluid and least rapidly from pericardial fluid

Chloride values for body fluids of human subjects dead of drowning in fresh water are shown in table 12 Although it was stated that none of these individuals had been dead longer than 6 days the absence of more definite information concerning the time of death makes it difficult to assess the significance of the

TABLE 10

*Chloride concentration of blood of human subjects probably dead of drowning in salt water (milli-equivalents per liter)*

| POSTMORTEM INTERVAL | WHOLE BLOOD | | | SERUM | | | REPORTED BY | POSTMORTEM INTERVAL | WHOLE BLOOD | | | SERUM | | | REPORTED BY |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | RH | LH | L-R | RH | LH | L-R | | | RH | LH | L-R | RH | LH | L-R | |
| *days* | | | | | | | | | | | | | | | |
| ½ | 102 | 114 | 12 | 118 | 137 | 19 | Moritz and McLean | Unspecified | 86 | 95 | 9 | | | | Gettler |
| ¾ | | | | 92 | 113 | 21 | Moritz and McLean | Unspecified | 96 | 105 | 9 | | | | Gettler |
| 1 | | | | 109 | 134 | 25 | Moritz and McLean | Unspecified | 111 | 124 | 13 | | | | Gettler |
| 3 | 98 | 139 | 41 | 97 | 139 | 42 | Moritz and McLean | Unspecified | 99 | 103 | 4 | | | | Gettler |
| 4 | 64 | 91 | 27 | | | | Gettler | Unspecified | 81 | 81 | 9 | | | | Palmer and Doherty |
| 5 | | | | 87 | 87 | 0 | Moritz and McLean | Unspecified | 81 | 100 | 19 | | | | Palmer and Doherty |
| 6 | 43 | 65 | 22 | | | | Gettler | Unspecified | 100 | 111 | 11 | | | | Palmer and Doherty |
| Unspecified | 41 | 47 | 6 | | | | Gettler | Unspecified | 112 | 116 | 4 | | | | Palmer and Doherty |
| Unspecified | 79 | 100 | 21 | | | | Gettler | Unspecified | 88 | 128 | 40 | | | | Palmer and Doherty |
| Unspecified | 105 | 116 | 11 | | | | Gettler | Unspecified | 71 | 81 | 10 | | | | Palmer and Doherty |
| Unspecified | 115 | 167 | 52 | | | | Gettler | Unspecified | 91 | 176 | 85 | | | | Palmer |
| Unspecified | 105 | 125 | 20 | | | | Gettler | Unspecified | ? | ? | 34 | | | | Palmer |
| Unspecified | 107 | 114 | 7 | | | | Gettler | Unspecified | ? | ? | 34 | | | | Palmer |
| Unspecified | 95 | 103 | 8 | | | | Gettler | Unspecified | ? | ? | 15 | | | | Palmer |
| Unspecified | 111 | 126 | 15 | | | | Gettler | Unspecified | ? | ? | 17 | | | | Palmer |
| Unspecified | 90 | 96 | 6 | | | | Gettler | Unspecified | ? | ? | 33 | | | | Palmer |

analytical results Chloride levels of pleural fluid as low as 76 mEq/L had been observed in the control series, and in 5 of the 11 cases of fresh water drowning, in which pleural fluid was tested, chloride levels lower than 76 were encountered The lowest chloride level observed in the peritoneal fluid in the control series was 67 mEq/L and in 3 of the 7 drowned persons chloride values lower than 67 were encountered The lowest chloride level observed in the pericardial fluid in the control series was 84 mEq/L and in 2 of 9 drowned persons lower values were encountered It is obvious that the control data are as yet inadequate to draw any conclusions concerning the significance of the chloride content of pleural, pericardial, peritoneal or pulmonary fluid in the diagnosis of death by drowning

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method determination of deaths by drowning

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time values 8 to 10 times as high as the antemortem levels were reached As in the case of postmortem chloride reduction the postmortem increase of magnesium in the blood does not always occur at the same rate in the two sides of the heart and differences between right and left heart as great as 0 7 mEq/L were observed

After drowning in sea water there was an early and sharp rise in the serum magnesium concentration on the left side of the heart to levels considerably higher than had been encountered in any of the control animals short of putrefaction Differences between the right and left heart's blood of animals drowned in salt water as great as 16 mEq/L were encountered

The highest serum magnesium values observed by Moritz and McLean in 6 human subjects dead less than 72 hours of causes other than drowning was 7 mEq/L, whereas in 2 or 3 cases of salt water drowning examined within 24 hours after death serum magnesium values in the left heart of 22 and 23 mgm per liter respectively were found The greatest difference in the magnesium content of serum from the right and left sides of the heart of persons dead of causes other than drowning was 0 7 mEq/L and differences as high as 8 mEq/L were observed in all three of the cases of salt water drowning in which the blood was analyzed for magnesium

If subsequent data concerning the rate and character of the postmortem changes in the magnesium content of blood conform to those now available it would appear that a preponderance of magnesium in the blood from the left side of the heart in excess of 1 mEq/L would constitute presumptive evidence of drowning in salt water

*Absence of significant chemical change in the blood of persons presumably dead of drowning* As already indicated the absence of chemical evidence of drowning can frequently be explained on the basis of postmortem artefact Occasionally, however, and even though the autopsy is performed within a few hours of death, it is found that a person without demonstrable disease has unexpectedly died in the water without inhaling a sufficient amount of water to be recognized in the air passages, to produce changes in the lungs, or to cause significant alteration in the chemical composition of the blood In such instances neither pathological nor chemical examination is likely to aid materially in establishing the cause of death If drowning be defined as suffocation due to the inhalation of fluid such deaths might better be characterized as having resulted from syncope Although it is beyond the scope of this review to discuss the possible mechanisms of such deaths it is pertinent to call attention to their occurrence There are no reliable data from which the frequency of their occurrence can be estimated It is the author's impression that unexplained (not due to inhalation of fluid) deaths in the water constitute considerably less than 10 per cent of all of the so-called deaths by drowning

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# THE RÔLE OF THE ADRENAL CORTEX IN PHYSIOLOGICAL PROCESSES

W W SWINGLE AND J W REMINGTON[1]

*Section of Physiology, Biological Laboratory, Princeton University*

The volume of literature dealing with various aspects of adrenal cortical function renders any review necessarily incomplete, both with respect to topics treated, and to references cited This paper is restricted to a discussion of but certain features of cortex function and the reviewers have arbitrarily omitted reference to the interrelationships between the cortex and other endocrine glands, and to histological changes which the adrenal undergoes under various experimental conditions Much of this literature has been covered in recent reviews (142, 460, 429, 274, 276, 211, 510)

No attempt has been made to survey work done prior to 1930 since this has been done by Britton (29) However, a few articles which cover the main trend of adrenal cortex physiology up to that time are included in the bibliography (425, 426, 12, 16, 134, 424, 182, 171, 164, 167, 342, 446, 447) Purely chemical problems concerned with preparation of cortical extracts and the isolation and identification of the various crystalline adrenal hormones also lie outside the province of this paper, and have been adequately discussed in several reviews (235, 236, 341, 399, 350, 351, 74) Probably most adrenal steroid chemists are agreed that not all of the hormones of the cortex have been isolated, for the amorphous fraction, which remains after the known steroid hormones have been removed from extract concentrates, is very potent in so far as life maintenance is concerned (235, 341, 249, 351)

For purposes of physiological treatment, the hormones of the adrenal cortex can be classified into two groups 1, those closely allied to progesterone the most active of which is desoxycorticosterone, and 2, the steroids which have an oxygen at the C-11 position, either in keto or hydroxy form, whose nomenclature is based on their relation to corticosterone The physiological activity of these latter hormones, while showing quantitative differences, is essentially similar, and seems primarily concerned in the regulation of some aspects of organic metabolism as opposed to inorganic For sake of brevity we shall refer to this group as the corticosterones, except where it is necessary to discriminate between its members Desoxycorticosterone is apparently the most potent of all known cortical hormones in maintaining the life of adrenalectomized animals, and in the control of certain phases of electrolyte metabolism Yet it is present in but minute quantities in the adrenal, so that experimental results based on its use may eventually prove atypical

A *Factors which affect the life span after adrenalectomy* Most adrenal work has been done on the dog, cat or rat The guinea pig and rabbit present operative difficulties which preclude their extensive use For many years it seemed

[1] Upjohn Research Fellow

that in the rat, unlike the other forms, adrenalectomy was fatal in only a fraction of the cases The current view is that widespread accessory adrenal bodies may take over the function of the extirpated glands in this form, although such accessory bodies have not been clearly demonstrated to be frequent enough to maintain life The mortality rate apparently depends in part on the strain of the animal (119, 121, 56) Removal of considerable surrounding tissue, said to be rich in accessory tissue, along with the adrenal glands, will increase the mortality (112, 339, 106, 142) If the animal is tided over the first few days following operation, thus presumably allowing time for accessory body hypertrophy, the mortality is decreased (129)

In the dog, the surgery involved in adrenal removal must be carefully performed, for injury to nerve plexuses adjacent to the glands will precipitate a rapid circulatory failure (243, 114) Adrenalectomy is therefore usually done in two stages (364, 114), a week or ten days elapsing between operations This is unnecessary, however, if spinal anesthesia is employed (243, 106), or thorough procaine infiltration of the gland and adjacent nerve elements is performed previous to extirpation (243)

Young rats tend to have a shorter survival span than older animals (250, 106, 491, 418) Although there is some dispute on the point, the sex of the animal probably has no great influence on survival (364, 142, 418) In hibernating forms the season of the year materially affects the life span, the animals generally surviving the period of torpor (30)

The diet is extremely important in an assay based on the survival of adrenalectomized animals The chief factor seems to be the intake of sodium chloride (373, 269, 159, 251, 128, 359, 491, 2, 6, 47), and the ratio of fed sodium to potassium For reasons not clearly understood, a diet containing bread will increase the survival time of adrenalectomized rats (430, 56, 59) As will be discussed more fully later, environmental temperature must be carefully controlled

It was first demonstrated by Rogoff and Stewart that the pregnant female dog (366) could survive removal of both adrenal glands Further work showed that pseudopregnancy would also alleviate the symptoms of adrenal insufficiency (367, 445, 62) Whereas administration of estrogens shortens the survival period (45, 73, 403, 340), apparently anterior pituitary hormones (92, 45) and the corpus luteum hormone, progesterone, (122, 125, 386, 107, 494, 77, 139, 68, 91, 340, 67) can maintain life

Many studies of assays for adrenal cortical hormones, based on the maintenance of life in adrenalectomized animals, have been published, for the dog (157, 343, 482), the cat (183), guinea pig (416, 381, 47) and the rat (188, 339, 418, 385, 44, 26, 78, 143) By their use, each steroid hormone has been tested In general, desoxycorticosterone is the most potent (466, 298, 143, 91, 248, 340, 58, 357, 47), although the amorphous fraction is almost comparable (237, 238, 249) Larger amounts of the corticosterones are required (235, 248, 298)

B *The relation of the adrenal cortex to electrolytic metabolism* 1 *Sodium metabolism* Baumann and Kurland (16) first called attention to a fall in plasma sodium and chloride, and a rise in potassium, which followed adrenalectomy in

cats, and expressed the opinion that the adrenal was concerned in sodium metabolism Later it was shown that the injection of various sodium salts (292) or large amounts of Ringer's solution (365) would greatly prolong the life-span of adrenalectomized animals, and even revive such animals from acute insufficiency

The importance of these observations was not fully realized until Loeb (268) showed that a significant decline in plasma sodium was characteristic of crisis in Addison's disease, and that the patient could be kept symptom free by the administration of large amounts of sodium chloride (269) In careful metabolic experiments, Loeb and associates (273, 275) and, later, Harrop and co-workers (158) demonstrated that the decline in plasma sodium levels in the adrenalectomized dog was a reflection of an abnormally large excretion of this ion by the kidney Adequate amounts of cortical extract restored the electrolyte pattern of the blood to normal, and prevented the renal wastage of sodium and chloride (156, 154, 158)

That sodium salts alone can maintain the life of adrenalectomized animals has been adequately demonstrated Swingle and associates (450) were only partially successful in increasing the life-span by feeding sodium chloride to the point of tolerance Harrop and co-workers (159) showed, however, that adrenalectomized dogs could be maintained for long periods if a sodium bicarbonate-sodium chloride mixture was given by stomach sound Allers (1) and Allers and Kendall (2) devised the most successful therapy for maintaining adrenalectomized dogs, based on the feeding of sodium chloride–sodium citrate mixture with a diet low in potassium Dogs were maintained on this regime, without cortical extract, for as long as 115 days, with normal values for all blood constituents When the salt therapy was discontinued, the animals rapidly developed adrenal insufficiency Cleghorn and associates (53) have confirmed these findings With careful control of the mineral constituents of the diet, an adrenalectomized dog receiving no other form of treatment can be maintained without food for as long as seven days, which is a most severe test (237)

Sodium chloride administration will also maintain the adrenalectomized rat as shown first by Rubin and Krick (378) and Gaunt (128) Very large amounts of sodium chloride, however, may be injurious, and lead to death of the animal (8)

Stahl and associates (421) successfully treated numerous Addison's disease patients with sodium chloride alone, and Harrop and associates (163), on the basis of their own work and that of Loeb, suggested the use of a salt-free diet as a diagnostic test for patients suspected of having this disease The evidence cited conclusively demonstrates that an adequate salt therapy can maintain normal health and normal blood chemistry both in adrenalectomized animals, and in patients with Addison's disease, provided the individual is not subjected to unusual stress of any kind (236, 237), and so long as the food intake remains normal (277)

The principal deficiency of the adrenalectomized animal with regard to sodium metabolism seems to lie in an inability of the tubular cells of the kidney to reab

sorb the ion from the glomerular filtrate (152) One theory advanced to explain this renal failure was that increased sodium excretion was related to a decreased ability of the kidney of the adrenalectomized animal to produce ammonia (227), but this view has not been generally accepted

When cortical extract is injected into the patient with Addison's disease, the normal human subject, the adrenalectomized dog, or the intact dog, the excretion of sodium is decreased, and that of potassium increased (464, 471, 180, 181) The sodium excretion is closely proportional to the dosage given (155, 160), which renders this technique valid for a bioassay of cortical materials

The most potent adrenal hormone possessing an action causing renal retention of sodium is desoxycorticosterone (468, 474, 237, 493, 274, 247, 470) In some cases the action of this steroid on sodium excretion is so powerful that serum sodium concentrations may actually be elevated above normal (101, 348), and the serum potassium levels lowered to the point at which toxic symptoms appear (247, 101)

The corticosterones are less active in causing sodium retention, or, as in the case of 17-hydroxy-11-dehydrocorticosterone, actually increase the sodium loss (469) However, the renal effect is not specific for adrenal cortical hormones, since the crystalline sex hormones also cause sodium retention (472, 473, 154, 155, 239) Estradiol and progesterone are the most active of the sex hormones in this respect, testosterone the least active Similar effects are obtained in the adrenalectomized animal, so that the renal action of the sex steroids is not mediated through the adrenal (467)

Hartman and his collaborators have shown that the action of cortical extract on sodium retention is gradually lost when injections are made intravenously over a period of time (165, 178, 180) This state of refractoriness does not develop when the extract is given subcutaneously (177) Refractoriness can be conferred upon a non-injected normal animal by the injection of serum from a refractory animal (477) However, the refractory state is not induced by injections of corticosterone, desoxycorticosterone or the precipitated sodium factor (173) Hartman and Lewis conclude that the factor necessary for sodium retention normally exists in a haptene combination with a protein contained in cortical extracts (173) It is apparently not identical with the factor which will maintain life in the adrenalectomized animal (176, 186, 185)

Absorption of electrolyte from the lumen of the intestine is distinctly abnormal in adrenalectomized animals (82, 422, 48), indicating that extra-renal defects may also be contributing to the impaired sodium metabolism

Increases in intracellular hydration which should accompany depletion of extracellular sodium and chloride by renal wastage, have been repeatedly demonstrated in adrenalectomized animals (414, 411, 507, 344, 314) Muntwyler and associates (314) were able to correlate the gain in muscle water with the decline in extracellular sodium concentrations The red blood cells show an increase in water content similar to that shown by muscle (194, 314)

Since an increased hydration of muscle and red blood cells also follows a lowering of extracellular electrolyte concentrations in the intact animal (81, 338,

521, 88, 314, 301), it can hardly be regarded as a specific response to lack of cortical hormones Winter and Hartman (507) found, however, that skeletal muscle taken from adrenalectomized rats would gain water more rapidly in a hypotonic solution, and lose it more rapidly in a hypertonic solution than would tissue from intact rats They attributed this to increased permeability of the muscle cells. Ponder and Gaunt (344) obtained negative results in similar experiments on rat muscles, but Angerer and Angerer (9) found the same changes noted by Winter and Hartman in muscles from adrenalectomized frogs

As suggested by Swingle and associates (432), the actual hemoconcentration and fall in sodium concentration of the blood is often greater than that which can be accounted for by renal loss of water and sodium or by a shift of fluid into the intracellular compartment Changes in membrane permeability are therefore suggested It is now rather firmly established that the adrenalectomized animal in insufficiency does show intracellular electrolyte concentration changes Voluntary muscles (194, 325, 153, 80, 314, 41, 51), cardiac muscle (80) and red blood cells (194) all show an increased potassium and a decreased sodium concentration Liver cell potassium remains unchanged (80) These changes can be corrected by cortical extract injections, but similar shifts of ions in intact animals receiving cortical extract have not been obtained Desoxycorticosterone injections will, however, strikingly alter the cation distribution in muscle cells Muscle sodium is increased in both adrenalectomized and normal rats after prolonged treatment with this steroid (41, 306, 101) and intracellular potassium is reduced

The intracellular electrolyte changes may not be reflecting a specific effect of adrenal cortical hormone upon membrane permeability, however Nephrectomized rats show intracellular potassium increases similar to those of adrenalectomized animals, as the plasma level rises, indicating that the intracellular concentration is directly governed by the extracellular potassium level (80) Red blood cells of the normal animal (149, 150, 242) respond to changes in sodium and potassium concentrations of the plasma by shifts of water and of these ions across the cell membrane When the cat or rabbit is subjected to depletion of extracellular sodium by intraperitoneal glucose injections, the red blood cells lose sodium into the plasma (361) Heppel (196) found that the muscles of the intact rat would gain sodium and lose potassium when the animal was fed a potassium free diet On the other hand, potassium injections will increase muscle potassium and lower muscle sodium (307) Thus while it is true that electrolyte shifts into and from the intracellular compartment occur in adrenal insufficiency, they apparently coincide with a fall in plasma sodium and a rise in plasma potassium concentrations, and are of the same magnitude as the intracellular changes exhibited by intact animals with similar extracellular electrolyte changes

The observed reduction in intracellular sodium contradicts the assumption previously mentioned, that a plasma sodium loss greater than that which can be accounted for by excess renal excretion, can be attributed to a shift into the tissues

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centrations to those typical of terminal adrenal insufficiency would reproduce many of the symptoms of the adrenalectomized animal, and might lead to death (189, 481, 530, 528, 531)

The therapeutic value of a diet low in potassium in the treatment of Addison's disease or for the maintenance of adrenalectomized animals has been repeatedly demonstrated (1, 2, 3, 325, 498, 378) Potassium administration has, in fact, been successfully used as the basis for a test for incipient adrenal deficiency in the human (76, 529)

Harrop, Soffer, Ellsworth and Trescher (158) first showed that the increase in plasma potassium concentrations was largely a reflection of a decreased renal capacity to excrete the ion This kidney failure could be corrected by cortical extract (154, 158) Harrison and Darrow (152) attributed the renal dysfunction to a disturbance in tubular function, so that potassium was not concentrated in the urine in a normal manner This potassium retention may not hold true for exogenous potassium, at least in the rat (380)

Not all investigators agree that renal failure will account for the whole of the plasma potassium increase found in adrenal insufficiency Marenzi (290) found that injected potassium was fixed by the tissue cells less readily in adrenalectomized animals Winkler and associates (505) also found that plasma potassium levels were elevated by a smaller amount of injected potassium than that required for the intact dog Cortical extract is, according to Marenzi, concerned in binding potassium in the tissue cells, in regulating the potassium equilibrium between tissues and plasma, and in regulating the excretion of excess plasma potassium Ingle, Nilson and Kendall (219) observed that cortical extract would retard the rise in potassium concentration and prolong life in the adrenalectomized nephrectomized rat Other investigators using this type of animal have reported either negative or inconclusive results insofar as potassium changes are concerned (284, 172)

Desoxycorticosterone has an action upon potassium metabolism even greater than that of cortical extract Long continued administration of this steroid may lead, in the dog, to muscle weakness and intermittent paralysis, which is in large part attributable to the extremely low plasma potassium concentrations found (247, 101) The symptoms can be readily prevented by potassium injections. The use of a low potassium diet with desoxycorticosterone therapy in patients with Addison's disease may have serious consequences (478) The striking effect of desoxycorticosterone upon potassium metabolism is probably largely upon the kidney, for the steroid will cause an increase in potassium excretion even in the intact animal (247) However Talbott and co-workers (458) found quite low potassium clearances in patients with Addison's disease even after long periods of desoxycorticosterone therapy

Desoxycorticosterone markedly affects the passage of potassium into and out of the cells It will prevent the usual increase in intracellular potassium which accompanies adrenal insufficiency, and will actually lower the tissue potassium in intact animals (306, 101, 41) Myocardial fibers of the heart show lesions

comparable to those observed in rats fed a potassium-free diet (306) This effect in reducing muscle potassium is also shown to some extent by the sex steroids (305)

The accumulation of potassium in muscle cells during adrenal insufficiency probably will not account for the asthenia characteristic of this condition (306) Similarly, the toxic effects of injected potassium appear to be directly related to elevation of the serum concentration, and only indirectly to the rise in muscle potassium (307) On the other hand, Ferrebee and associates (101) have suggested that the muscle weakness which follows prolonged treatment with desoxycorticosterone may be associated with the decreased muscle potassium, and its replacement with sodium

The idea that accumulation of potassium in the serum may, in itself, account for the symptoms of adrenal insufficiency has not had wide acceptance Keith and Binger (232) report that induced high levels of serum potassium in normal human subjects, and equally high levels in diseased patients, do not necessarily produce toxic symptoms Schamp (382) was unable to obtain symptoms resembling those of adrenal insufficiency in normal dogs injected, for long periods, with potassium salts The patient with Addison's disease may show little or no potassium retention even in crisis, and, on the other hand, may show greatly elevated serum potassium levels and yet remain symptom-free (271) Adrenalectomized animals dying of circulatory failure after trauma show no consistent serum potassium change (444, 355)

Neither can the cause of death following adrenalectomy be attributed solely to the upset in the metabolism of sodium, although this was at one time a rather common assumption (273, 272, 158) While renal wastage of salt and water and consequent dehydration certainly contribute to the fatal collapse of the adrenalectomized animal, a considerable body of evidence indicates that these changes, *per se*, may not be the primary factors involved Experiments have been devised in which the serum electrolyte concentrations of adrenalectomized animals are reduced to levels as low as or lower than those characteristic of adrenal insufficiency, without producing any symptoms whatever Such electrolyte depleted animals can be run through complete cycles of circulatory failure and subsequent restoration to normal health and vigor by withholding or administering cortical extracts (440, 442) Moreover, circulatory collapse in adrenalectomized dogs need not involve any change in blood sodium or in body water (444, 355) Intravenous injections of a strong hypertonic salt solution can restore the failing circulation and electrolyte concentrations of the dog in acute adrenal insufficiency, but the effect is temporary Within a few hours the blood pressure again declines to a level incompatible with life, and the animal may die with serum electrolytes still well elevated above normal (439, 443)

Hartman, Lewis and Gabriel (175, 176) reported that adrenalectomized dogs rendered refractory to cortical extract insofar as renal retention of sodium is concerned can be maintained in apparently normal health by extract injections, while the plasma sodium is at the level characteristic of adrenal insufficiency

Loeb (270) has reported cases of Addison's disease where the electrolyte pattern of the blood is virtually normal at death.

Some animal forms, such as the opossum (36, 412, 187) and the elasmobranch (174) show either no decrease or even an increase in plasma sodium during adrenal insufficiency

Further evidence on this point is furnished by experiments employing the intraperitoneal injection of isotonic glucose (383, 81) Intact animals so treated show symptoms resembling, in many respects, those characteristic of adrenal insufficiency, which include low serum sodium concentrations, hemoconcentration, and decreased tolerance to stress (131, 361, 314) As has been frequently demonstrated, the adrenalectomized animal is extremely sensitive to intraperitoneal glucose injections (439, 154, 124, 515, 352)

In the adrenalectomized dog, the depletion of extracellular electrolyte and the dehydration of the blood is followed by a progressive decline in blood pressure and symptoms of acute circulatory collapse (439) Qualitative differences in the electrolyte transfers induced in adrenalectomized and intact animals by intraperitoneal glucose injection have not been observed Circulatory failure can be prevented by either cortical extract or desoxycorticosterone (435), even though plasma electrolyte concentrations remain low (440) Blood chemistry studies made on adrenalectomized rats subjected to intraperitoneal glucose injections have revealed *a*, that less electrolyte is shifted into the peritoneal cavity than in the normal rat, *b*, that, despite this smaller shift, serum electrolyte concentrations actually fall lower than in the normal rat, and *c*, the blood concentration is more severe (352) Either cortical extract or large amounts of desoxycorticosterone can prevent the fatal collapse, but neither will correct in entirety the abnormalities in electrolyte transfer (353) However, the electrolyte imbalance does not seem directly concerned in the production of circulatory collapse, except insofar as it throws a strain upon an asthenic peripheral circulation

The mechanism underlying the decreased ability to transfer fluid and electrolyte from one body compartment to another is not clear Cantarow and Rakoff (42, 349) found that desoxycorticosterone, in common with the sex hormones, increased the rate of chloride transfer from the blood to the peritoneal cavity in the dog, but a similar change is not observed in the rabbit (349) or rat (353)

C *The relation of the adrenal cortex to renal function* Aside from renal wastage of salt and water, and retention of potassium, in adrenal insufficiency, the adrenalectomized animal shows changes in blood and urine chemistry which are indicative of a serious impairment in renal function There is an early rise in blood non protein nitrogen and urea nitrogen levels (23, 12, 414, 433, 434, 279, 182, 520, 160, 525), and an appreciable increase in blood sulfate (455, 509), creatinine, uric acid and phosphate levels (434, 522, 182, 160) In 1916 Marshall and Davis (297) showed that the urea retention of the adrenalectomized dog was associated with a decreased excretion of creatinine and phenolsulphonphthalein A decreased urea clearance was also noticed by Bevier and Shevsky (23) in the

adrenalectomized rabbit While a more or less acute lipoid nephro: kidney tubules in terminal adrenal insufficiency has been described 146, 417) it now seems agreed that visible pathological changes ar consistent nor widespread enough to explain the impairment in kidney (433, 130, 297)

Stahl and associates (421) showed that the clearance of either urea o sulphonphthalein was not reduced below normal in adrenalectomized a: long as cortical extract or salt was given, but that lowered clearances shortly after the removal of maintenance therapy A close correlation repeatedly drawn between the actual level of non-protein nitrogen in t and the onset of symptoms of adrenal insufficiency (520, 182, 160, 4 The possibility that death in the adrenalectomized animal is due pri uremia is not tenable, however, for, as several workers have demonstr. 300) blood urea nitrogen levels higher than those usually observed in adrenal insufficiency are not necessarily lethal to the animal with intac The blood urea changes are, however, indicative of a progressive rena which must be regarded as an essential factor in the development of u cated adrenal insufficiency

Distinct deviations from normal in the renal excretion of nitrogeno products, of sodium, of potassium, and of water are now recognized a: teristic of adrenal insufficiency At present, these various deficiencie seem to have a common denominator

The inability of the kidney of the adrenalectomized animal to transfe from the glomerular filtrate to the plasma, and also its inability to excre sium in normal quantities, have already been discussed Both are su due to a defect in the metabolism of the renal tubules, but one which ca rected by desoxycorticosterone (468), and the amorphous fraction (238 it is presumably not directly linked to a disturbance in intermediary carb metabolism

The decreased excretion of nitrogenous waste products in the adrenale animal is probably related to extra-renal defects The increase in bl nitrogen following adrenalectomy in the dog occurs simultaneously fall in blood pressure, and does not precede it (449) In fact, there is reciprocal correlation between the blood pressure and blood urea Talbott and associates (458) have recently shown, however, that a defic the rate of glomerular filtration exists in the patient with Addison's dise when the blood pressure is well maintained, so that hypotension is cert the sole factor concerned The sharp reduction in blood volume whic reduction of sodium and chloride concentrations also must play a rôl creasing the renal blood flow in the adrenalectomized animal Talb associates (458) suggest that a diminution in efferent arteriolar tone ma significant part in the reduced urea clearance

Desoxycorticosterone will increase the urea excretion, and lower the blood urea levels (466, 274, 176, 377) In fact, the blood urea level of the animal can be lowered by administration of large amounts of this sterc

357) Not all of this difference can be attributed to an increased rate of glomerular filtration, for desoxycorticosterone will also decrease the severity of uremia in nephrectomized animals (398, 362, 85, 404) Other indirect evidence indicates that this steroid may actually depress protein catabolism of the body (357, 355)

D *Relation of adrenal cortex to water diuresis, water intoxication and diabetes insipidus* In most, but not all, animals, the altered electrolyte excretion which follows adrenalectomy is associated with a diuresis This subsides, as symptoms become severe, and terminally an oliguria or anuria ensues (162, 507, 440, 373, 19 380, 126, 274) Yet despite this water diuresis associated with sodium loss, the ability of the kidney to excrete water shows deficiencies If distilled water is administered by mouth, even in small doses, the diuretic response is far below normal (291, 118, 372, 260) and susceptibility to water intoxication is extreme (360, 441, 124, 98) This loss of the normal diuretic response to water is evident in the rat within 18 hours after adrenalectomy and later becomes more marked even in animals well maintained on salt (118) Protection against water intoxication is also afforded the adrenalectomized rat to a certain degree by salt (441, 124)

Kottke and associates (246) observed no abnormality in a urine dilution test performed on adrenalectomized dogs maintained on salt The ability to concentrate urine was defective, however, in hot weather

Desoxycorticosterone shows activity in preventing water intoxication in adrenalectomized animals (453, 98), but whole gland extract or the corticosterones (98) are more effective The sex hormones show no activity (125) Cortical extract and desoxycorticosterone will also provide a life-maintaining protection for normal rats against doses of water otherwise lethally intoxicating (118)

The mechanism underlying the loss of the diuretic response to water after adrenalectomy has not been fully elucidated, although several contributing factors are known In the rat, delayed diuresis after water is given by mouth can be accounted for in part by a decreased stomach-emptying time and rate of intestinal absorption (118) The decreased absorption rate is probably associated with an abnormal electrolyte shift into the intestinal lumen (118, 422) However, either absorbed water (118, 361a) or injected fluid (360, 241) is not excreted at a normal rate in adrenalectomized animals, or in patients with Addison's disease Further, intoxication symptoms appear at lower levels of water retention in adrenalectomized than in normal dogs (441) Water intoxication is apparently not similar to most other stresses in adrenalectomized animals since, at least in its early stages, it is not associated with a marked fall in blood pressure (441, 118)

The hypophysectomized animal also shows a striking loss of diuretic response to water and a high susceptibility to water intoxication (124, 228), which can be relieved by desoxycorticosterone or cortical extract (228)

Large doses of desoxycorticosterone, when prolonged over a sufficient time interval, will produce a syndrome of polydipsia and polyuria similar to that of diabetes insipidus (348), except that pituitrin is ineffective and fluid restriction

does not cause dehydration Withdrawing salt from the diet reduces the severity of the polyuria (312) An imbalance between the effect of the adrenocortical and posterior pituitary hormones upon the kidney has been postulated (312, 413) It is true that desoxycorticosterone (70, 400), unlike cortical extract (388), will produce a diuresis in hypophysectomized rats However, the quantities of the steroid required to produce polyuria are so large that the effect may be of the nature of a simple overdosage phenomenon (387)

Another explanation for the polyuria following desoxycorticosterone therapy might be that as the extracellular sodium concentration is increased, and intracellular hydration is decreased, the water intake, through increased thirst, will also be considerably increased (348, 356, 132, 81) In other words, the polyuria would be merely reflecting a polydipsia

E *Influence of the adrenal cortex on organic metabolism* The observation that the blood sugar level of adrenalectomized animals was often low, or even at hypoglycemic levels, was reported many years ago (24, 345) Later it was shown that the liver glycogen levels were even less stable than were the blood sugar concentrations (345, 71) but these important findings attracted little attention at the time Britton and his colleagues (32, 34, 33, 415) in a survey of the symptoms which follow bilateral adrenalectomy, in a wide variety of species, were impressed with the relative constancy of an appreciable decline in body carbohydrate levels, evidenced by blood sugar, and liver, heart and muscle glycogen changes The occasional entire lack of significant decline in blood electrolyte concentrations, and the frequency of the development of hypoglycemia, seemed to warrant the conclusion that death from adrenal insufficiency was a reflection of a faulty carbohydrate metabolism of the tissues of the body through depletion of carbohydrate levels

However, many observers pointed out that the blood sugar might easily be in the normal range even at the time of fatal collapse (161, 330, 141, 364, 12) which led some to doubt that regulation of carbohydrate metabolism was the prepotent function of the adrenal cortex

Several reasons for this apparent discrepancy in experimental results now seem clear There appears to be a rather wide species difference in the tendency for the blood sugar to decline significantly after adrenalectomy In the adrenalectomized cat, for example, the final collapse of adrenal insufficiency is often associated with a definite hypoglycemia (34, 528, 433, 33) The adrenalectomized dog, however, but rarely shows a decline in blood sugar levels (161, 330, 364, 12, 182) A part of this species difference is probably explicable on the basis of differences in eating habits

The problem of the relation of the adrenal cortex to carbohydrate metabolism was reopened with the work of Long and associates (277) In agreement with other workers, they found that so long as adrenalectomized rats were maintained in good health by the administration of sodium salts, no abnormalities could be observed in the storage of carbohydrate When, on the other hand, the adrenalectomized animal refused food, or when it was forced to fast, the liver glycogen showed a dramatic fall to extremely low levels, accompanied by less severe but

significant declines in muscle glycogen and blood sugar values Not only could these changes be prevented by the administration of cortical extract, but the liver glycogen levels could be increased well above normal even in these fasted animals, thus confirming previous observations of Britton and co-workers (34)

An increase in liver glycogen levels in the fasted animal, either normal or adrenalectomized, indicated that the administered cortical extract was either inducing a marked shift in carbohydrate reserves from other tissues of the body to the liver, or that the extract was stimulating the conversion of other materials into liver glycogen The fact that all known glycogen deposits were increased contradicted the possibility of a carbohydrate translocation after extract treatment In careful metabolic experiments, it was observed that the increase in liver glycogen levels was accompanied by an increase in urinary nitrogen excretion, with the ratio of extra carbohydrate formed to extra nitrogen eliminated indicating that the glycogen increment could be entirely explained by a conversion of endogenous protein stores to carbohydrate Coincident with the increase in nitrogen output there was a fall in body weight, without a comparable increase in metabolic rate

Evidence advanced by several investigators tends to support this general thesis that the action of cortical hormone may be more concerned with the catabolism of protein than in the actual utilization of carbohydrate Not only will adrenalectomy relieve the symptoms of severe diabetes in the pancreatectomized animal (108, 278, 203, 280), but, as the glycosuria is reduced and finally eliminated, the level of urinary nitrogen also decreases (277, 220, 278) Cortical extract administration will increase the degree of glycosuria and also increase the urinary nitrogen When phlorhizin is given to the adrenalectomized rat, the excretion of glucose and nitrogen is but a fraction of that observed with the phlorhizin treated normal animal (430, 199, 94, 264, 492) The administration of cortical extract produces a marked loss in body weight, increases the glycosuria and also the amount of nitrogen excreted Likewise the rise in liver glycogen, accompanied by an increase in urinary nitrogen, which follows a short period of anoxia, is prevented by adrenalectomy (94, 108, 265, but see 253), and can be restored with cortical extract injections

While this relation of the urinary nitrogen excretion to the changes in liver glycogen level appears clear, it should be remembered that a decreased nitrogen output, accompanied by an elevation of blood non protein nitrogen concentrations, is one of the earliest signs of adrenal insufficiency and denotes a decreased urea clearance by a hypofunctional kidney (151, 227, 158)

It should not be inferred that a regulatory effect of the adrenal cortex upon the catabolism of protein can satisfactorily explain all the metabolic deficiencies observable in the adrenalectomized animal The rapidity with which the liver glycogen level declines in a fasted adrenalectomized animal is an indication that the tissues are oxidizing carbohydrate at an abnormally rapid rate Evans (95) noted that when an adrenalectomized rat was fed glucose, it stored less as liver glycogen and used a greater portion than would an intact rat Conversely, Russell (375) found that the administration of cortical extract to a glucose fed rat

increased the proportion stored as glycogen, and decreased the amount oxidized by the tissues Katzin and Long (230) found a lower R Q in such extract treated rats The conclusion that abnormal amounts of carbohydrate are burned by the adrenalectomized animal is supported by the experiments of Thorn and co-workers (264, 476) These facts might be interpreted as indicating that cortical extract actually inhibits the oxidation of carbohydrate When, for example, force-fed depancreatized rats are given cortical extract, the increased glucose excretion which follows cannot all be linked to protein catabolism (220) Also, the decrease in sugar excretion which follows adrenalectomy is not entirely related to the decreased conversion of protein to carbohydrate (220) An interesting possibility is the one suggested by Wells and Kendall (493), that cortical hormone specifically blocks the action of insulin It is true that the adrenalectomized animal is extremely sensitive to insulin, and that it can be protected by the administration of cortical extract (226) or adreno-cortical transplants (431) Whether this antagonism to insulin is other than an indirect reflection of a more fundamental upset in carbohydrate or protein metabolism is not proven, but research along these lines is likely to prove fruitful

It would seem that the precise rôle of the adrenal cortex in metabolism must await clearly drawn results obtained on a study of the intermediary processes by which carbohydrate and protein are utilized in the tissues themselves Some suggestions have already been advanced, but any attempt to evaluate them would be premature There are, however, several points at which cortical hormones could be acting in their influence on metabolic processes

1 *Intestinal absorption* The theory advanced by Verzar (485, 486, 487, 308, 309, 221) that the hormone of the adrenal cortex is essentially concerned in the maintenance of the enzymatic processes by which carbohydrate or fatty acids are phosphorylated, was largely based upon the observation that the absorption rate for glucose and fat was reduced immediately after adrenalectomy In fact, the adrenalectomized rat was reported to have lost the differential between the absorption rate of glucose, which is phosphorylated in the intestinal mucosa, and xylose, which is not The reduced intestinal absorption shown by the adrenalectomized animal was therefore comparable to that shown by the animal poisoned with iodoacetic acid (486, 221, 308)

Since the publication of this scheme, much evidence has been advanced which has, with little exception, failed to substantiate Verzar's claims It is true that the absorption rate of glucose is lessened in the adrenalectomized rat (71, 4, 296, 204) On the other hand, when the animal is maintained on sodium salts, the glucose absorption is normal (83, 4, 49) One reasonable explanation offered is that the maintenance of a normal absorption rate depends directly upon the maintenance of a normal food intake Fasted normal rats, sham operated and unilaterally adrenalectomized rats show a decreased glucose absorption rate of the same order as that found in adrenalectomized rats (296), so that changes present shortly after operation presumably reflect the period of inanition on the day of the operation Considerable indirect evidence supports this conclusion, for it has been shown that liver glycogen and blood sugar levels show little

tendency to decline so long as the adrenalectomized animal is in good condition and eating normally

The claim of Verzar and other workers (487, 256, 257) that the absorption rate of fat is retarded after adrenalectomy, and can be restored to normal with either cortical extract or flavine and phosphoric acid, has led to considerable controversy Some investigators have confirmed the decreased absorption rate (18, 17), while others deny that the salt-fed adrenalectomized rat shows any deficiency in fat absorption (13, 15, 50, 14, 304, 427) or in the phosphorylation of fatty acids in the intestinal mucosa (427) Whether this discrepancy may be due to differences in technique is not settled (14)

There is, however, insufficient evidence to justify the suspicion that an inability to absorb either carbohydrate or fat from the intestine plays a significant rôle in the metabolic upsets which accompany adrenal insufficiency

2 *The conversion of absorbed glucose to glycogen in the liver* Available indirect evidence indicates that fed carbohydrate can be transformed into liver glycogen at a normal rate in the adrenalectomized animal (277, 7, 236) However, Britton and Corey (31) have presented direct evidence that cortical extract affects the ability of the liver to convert glucose into glycogen The perfused liver of an adrenalectomized rat failed to show glycogen storage with a gum-saline-glucose perfusion medium, even when insulin was given When, however, cortical extract was added to the perfusion medium, there was a striking and rapid rise in liver glycogen levels

Seckel (394) had earlier shown that cortical extract would retard the rate of glycogenolysis in liver slices taken from adrenalectomized rats This decreased rate of conversion of glycogen to glucose under cortical extract action was confirmed by Corey and Britton (69) using isolated livers The conclusion would be that cortical extract not only enhances the conversion of glucose into glycogen, but retards the breakdown of liver glycogen to maintain blood sugar levels The importance of this shift in the equilibrium between glucose and glycogen in favor of the glycogen cannot be assessed at present It might explain the fact that cortical extract will increase the proportion of fed glucose which is stored as liver glycogen as against that which is oxidized by the tissues However, it seems doubtful if all the known deficiencies in carbohydrate metabolism shown by the adrenalectomized animal can be explained on the basis of this reaction alone

3 *Deaminization of amino acids* A faulty deaminization of amino acids in kidney or liver could well explain the decreased ammonia and urea excretion found in adrenalectomized animals, and also the parallel increase in urinary nitrogen which accompanies the increase in glycogen stores following the administration of cortical extract Such a failure of deaminization, at least for the kidney, has some experimental backing

Jiminez-Diaz (227), impressed with the frequency of acidosis in Addison's disease patients, advanced the explanation that the kidney had become "asthenic," and no longer produced ammonia at a normal rate Kidney slices from adrenalectomized cats showed just such a failure of deaminization Russell and Wilhelmi (376), in extending this work, showed that kidney slices from adrenalec-

tomized rats did not deaminize either administered alanine or glutamic acid at a normal rate Samuels and co-workers (379) had observed earlier that alanine fed to adrenalectomized rats resulted in less storage of liver glycogen than would be true of normal rats

It is curious that this failure of ammonia removal could not be demonstrated for liver tissue Evans (96) found a normal rate of deaminization for alanine in liver slices from adrenalectomized rats Likewise, Koepf and associates (244) found a normal deaminization of glutamic acid in liver tissue

The basis for this tissue difference in the ability to deaminize amino acids, is not clear At least, until this point is clarified, it would not be safe to attribute the principal deficiency in protein and carbohydrate metabolism shown by the adrenalectomized animal to a failure in the enzymatic process involved in the liberation of ammonia

4 *Conversion of keto and hydroxy acid to carbohydrate* Considerable evidence has accumulated that keto acids, such as those remaining after deaminization of amino acids, cannot be transformed into carbohydrate at a normal rate after the adrenals have been removed Buell, Anderson and Strauss (39) found a retarded conversion of lactic acid to glycogen in the liver of adrenalectomized rats Similarly pyruvate and succinate do not seem to be transformed into glycogen at a normal rate in liver tissue deprived of cortical hormone (476, 244) When the animal is treated with large amounts of cortical extract, the conversion rate of pyruvate to glycogen is actually increased to greater than normal (244) On the other hand, kidney slices from adrenalectomized rats evidently form carbohydrate from succinic and pyruvic acid at a normal rate (376) This again may be due to a difference in tissue source

There is also some evidence that the animal as a whole cannot form glycogen at a normal rate from fed keto acids after adrenalectomy Lewis and associates (264) found that in phlorhizin treated rats, the transformation of lactic and pyruvic acids into glycogen proceeded at a slower rate after adrenal removal, but their results have been criticized by Kendall (235) Wells and Kendall (494) believe that there is no diminution in the ability to convert fed protein into liver glycogen in the phloridzinized adrenalectomized animal Levy Simpson (263) has suggested that cortical extract increases the conversion of endogenous lactic acid to carbohydrate in the Addison's disease patient

5 *Oxidation of glucose* The previously mentioned experiments indicating that the adrenalectomized animal burns a greater amount of carbohydrate than does the cortical extract treated animal (95, 375, 264, 476) would not necessarily demand that the actual process of glucose oxidation was abnormal If, for example, the catabolism of other endogenous energy yielding materials, such as protein, was impeded in the adrenalectomized animal, the oxidation of a larger amount of carbohydrate would necessarily follow

The only direct evidence on the ability of the tissues from adrenalectomized animals to utilize carbohydrate has been obtained on voluntary muscle It has long been recognized that muscle from the adrenalectomized animal rapidly loses its normal capacity to perform work In fact, several accurate methods of bio-

assay of cortical principles are based on the restoration of the normal work capacity either of the whole animal (117) or of isolated muscles (212, 147, 97) Ingle, in a number of studies on the work capacity of the gastrocnemius muscle of adrenalectomized rats, has shown that asthenia develops shortly after extirpation of the glands, and that it is not corrected by injections of salt (213, 218) Injected glucose produces an improvement in the work capacity, approximately equivalent to that obtained by the injection of cortical hormone (218) Winter and Knowlton (508) attribute the asthenia to a metabolic deficiency of the muscle of the adrenalectomized animal The fundamental nature of the decreased work capacity is, however, not known, for factors such as circulatory collapse and the decreased muscle glycogen have not been eliminated (218)

Britton and his associates (32, 34, 35, 33) have held that muscle glycogen levels are without exception seriously depleted in adrenalectomized animals, and conversely, that these glycogen levels could be restored by the administration of cortical extract The present consensus of opinion is, however, that while muscle glycogen may show a decline after adrenalectomy only after food is refused (277, 375) muscle glycogen is not increased in the normal animal treated with cortical extract Whether a carbohydrate deficiency other than that attributable to a lack of available glycogen is present in the muscle is uncertain The working muscle does seem to produce less lactic acid when deprived of cortical extract (11, 315, 66) Also there is impairment of the ability of the gastrocnemius to produce lactic acid autolytically (40, 66) A decrease in total phosphorus and in inorganic phosphate following adrenalectomy has been reported (40) Some workers (252, 66) believe that there is a reduction in phosphocreatin, which would imply a derangement in the recovery process of muscle Others doubt the significance of the phosphocreatin changes after adrenalectomy (281) Cope and co-workers (66) could find no difference in the enzymatic formation of lactic acid by muscle extracts from normal and adrenalectomized animals They concluded, however, that less work could be performed because the muscle could no longer resynthesize phosphocreatin at a normal rate

Verzar and Nontigel (488) found a decrease in glycogen phosphorylation in muscle after adrenalectomy, but since restoration was obtained with desoxycorticosterone, which does not, to an appreciable extent, influence muscle work (215), their result is difficult to evaluate Nicholson and associates (323) showed that the ability to develop a delayed tension (5th stage of neuro-muscular transmission) after a motor nerve stimulation, was reduced after adrenalectomy and could be restored by desoxycorticosterone There is some evidence that the energy capacity of the normal dog (87), rat (148), human (205, 310) and asthenic patients (136) may be increased by cortical extract therapy

It is not unlikely, therefore, that a deficiency in work capacity is related in part to an inability of the muscle from an adrenalectomized animal to utilize efficiently available carbohydrate, but the nature of this deficiency remains elusive

6 *Production and combustion of acetone body residues* Just as adrenalectomy reduces the glycosuria of depancreatized animals, it also tends to lower or abolish the excretion of ketone bodies (278, 280) Similarly, adrenalectomy will abolish

the ketonuria following phlorhizin injections (95), pregnancy (282), fasting (282) and the administration of anterior pituitary extract (116, 283) The actual production of ketonuria by cortical extract is not so clearly established Ketosis following the injection of 17-hydroxy-11-dehydro-corticosterone was obtained by Ingle and Thorn (220) in depancreatized rats, but, as Ingle (211) points out, this followed the development of a glycosuria sufficiently severe to be accompanied by ketonuria itself Ketone body excretion is increased in phlorhizin treated adrenalectomized rats after treatment with corticosterone, but the condition of the animal is at the same time improved (235) If fat is fed the phlorhizinized animal, there is little increase in the degree of ketosis unless cortical extract is given (494) However, desoxycorticosterone, which seemingly lacks an effect on carbohydrate metabolism, is also effective in this regard Ketonuria could not be produced in normal rats with cortical hormones (407)

A direct relation of cortical hormone to acetone body production following anterior pituitary extract is rendered unlikely by the observation of Mirsky and associates (311, 317) that there is no parallel decrease in the blood level of acetone bodies of adrenalectomized animals accompanying the decrease in ketonuria This observation has since been confirmed (287, 408) An explanation would be that the hypofunctional kidney shows a higher threshold level for acetone bodies Shipley (406), however, disagrees that the diminished ketosis in adrenalectomized rats treated with anterior pituitary extract can be explained solely on the basis of renal threshold level In his experiments, ketonemia was reduced but not abolished, and ketonuria was likewise reduced but not necessarily abolished Mirsky and associates (317) would agree that adrenalectomy does produce a reduction in the rate of ketone body formation and in the rate of utilization

An interesting fact, of uncertain significance, is that the fatty infiltration of the liver which follows the administration of anterior pituitary extract (116, 282), or poisoning with phlorhizin (487) can be prevented by adrenalectomy Adrenalectomy will also reduce the fat deposition in the regenerating liver of partially hepatectomized fasted animals (285) Cortical extract treatment allows the fatty infiltration to progress normally

By way of summary, then, it would seem that cortical hormones may control carbohydrate metabolism by *a*, increasing the conversion to glycogen of fed carbohydrate, *b*, allowing the conversion of endogenous protein to glycogen, assisting either in the process of deaminization of the amino acids, or in the conversion of the keto and hydroxy acids to carbohydrate, *c*, decreasing the oxidation of available carbohydrate

In almost all cases where cortical extract has an effect on the physiological processes involved in carbohydrate and protein metabolism, identical or even greater effects can be obtained with the corticosterones, which can prevent the depletion of liver glycogen in fasted adrenalectomized animals (277, 137, 276), raise the resistance to insulin injections (137, 138), increase the work capacity of voluntary muscle (214), augment the conversion of glucose to liver glycogen (277) and increase the conversion of keto acids to liver glycogen (264) It is also true that the adrenal steroids lacking in oxygen at C-11, such as desoxy-

corticosterone, 17 hydroxydesoxycorticosterone, or the sex steroid progesterone (277, 235, 265, 215, 137), have little effect on any of these processes, although they may increase liver glycogen levels in some species (123, 72)

The ability to augment liver glycogen stores is not as specific for cortical hormones as once believed, however, for Janes and Nelson (224) showed that diethylstilboesterol would increase liver glycogen in the normal rat This observation has been amply confirmed not only for this synthetic hormone but for the naturally occurring estrogens as well (140, 84, 216, 217) Accompanying the increase in carbohydrate is a rise in urinary nitrogen Dohn, Joseph and Gaunt (84) found that estrogens would increase the glycosuria of depancreatized ferrets, and Ingle (216) reported that they would produce glycosuria in the sugar fed normal rat Long (276) found that diethylstilboesterol would not increase the liver glycogen level in either the adrenalectomized or hypophysectomized animal, and concluded that the action of the sex steroids was on the adrenal cortex via the anterior pituitary Ingle (217) was able, however, to produce glycosuria in the adrenalectomized rat receiving relatively small amounts of cortical extract by prolonged sex steroid therapy Death might follow with blood sugar levels still markedly elevated This problem remains, therefore, unsettled

The metabolic upsets in protein and carbohydrate metabolism found in the adrenalectomized animal are, in large part, present in less acute form in the hypophysectomized animal No attempt has been made to review the rather extensive literature pertaining to the rôle of the anterior pituitary on organic metabolism, but it seems apparent that all known carbohydrate effects of the pituitary cannot be explained solely on the basis of its action in maintaining the functional integrity of the adrenal cortex (277, 276, 94, 375, 374, 21, 202, 429)

Evidence on the relation of the adrenal cortical hormone to vitamin metabolism is controversial For example, evidence for (266, 267, 500) and against (483, 428, 144, 225) an influence on the synthesis of vitamin C has been presented The conclusion of Verzar and Laszt (485, 486, 257) that phosphorylated riboflavine could maintain the adrenalectomized rat, has been repeatedly denied (295, 46, 316, 37, 100)

A defect in the calcification of teeth (337, 384) and bones (501) is present in the adrenalectomized animal, but its nature is obscure Butcher (38) found that hair growth was accelerated after adrenal removal, which is opposite to the effect which might be expected on the basis of the hirsutism associated with adrenal tumors, and also the hair shedding which accompanies adrenal insufficiency in the dog The hair shedding following adrenalectomy in the rat has been attributed to loss of the medulla (423) It is common observation, however, that hair coat changes can be partially corrected in desoxycorticosterone maintained adrenalectomized dogs

F *The adrenal cortex and resistance to stress* An extensive list can be constructed of drugs, poisons, toxins, infections, altered environmental conditions, and traumatic procedures to which the adrenalectomized animal is highly susceptible Only those which have received greatest attention can be discussed here

1 *Drugs, poisons and other pharmacological agents* Work has centered chiefly upon two, morphine (389, 479, 286, 259) and histamine (79, 293, 513, 516, 209, 369, 326) For both, the toxic dose for adrenalectomized animals is but a fraction of that required for the intact animal The resistance can be readily increased by the injection of cortical extract, although it is questionable whether complete restoration to the normal is thereby attained and whether the adrenal medulla may not also play a part in the lowered resistance (513, 209), at least to histamine

The simplest explanation for the lowered resistance would be that the capacity to destroy or inactivate the administered drugs was impaired There is evidence that this may be the case for histamine (369) The capacity of histamine inactivation can be restored by cortical extract (368) and, less effectively, by desoxycorticosterone (368, 326)

If the secretion of the adrenal cortex is specifically involved in the inactivation of injected drugs, then it should be possible to enhance the resistance of the normal animal by cortical extract treatment Perla and associates (334) did find that cortical extract would increase the efficacy of a saline infusion in preventing shock after histamine injections The coupling of two therapeutic measures, either of which might be useful in treatment of shock, makes the results difficult to interpret Irradiation of normal dogs with carbon arc lamps produces a fall in blood pressure Graham (136a) observed that in adrenalectomized dogs the fall in diastolic pressure averaged 63 per cent Both cortical extract and desoxycorticosterone prevented the blood pressure fall in untreated normal and adrenalectomized animals The protective action of the steroid and extract was assumed to be due to the effect they exert against the toxic manifestations of histamine since carbon arc irradiation results in the release of a vasodilator substance from irradiated tissues

2 *Toxins, anaphylaxis, infections* The adrenalectomized animal is highly susceptible to but small amounts of diphtheria toxin (20, 197), typhoid vaccine (457), bacterial infection (390, 223), foreign cells (335, 336, 337) and foreign serum (109, 514) In acute adrenal insufficiency, the capacity to form antibodies is decreased (335, 337) although this may be attributable to loss of the medulla (336) The opsonic index of the adrenalectomized rat is reduced (26a) Cortical extract will restore the resistance to normal (184, 93) Desoxycorticosterone (93), unlike the corticosterones (235), is apparently ineffective against typhoid vaccine

If the adrenal cortex is to be regarded as essential for the immunity reaction, cortical hormone should afford protection to the normal animal against infections and against toxins Evidence on this point seems sharply divided Protection against diphtheria toxin (527), anaphylactic shock (512), tuberculosis (346), and infections (240, 336a, 496) have been reported in intact animals and in humans On the other hand, an effect against infections or toxins (392, 497), typhoid vaccine (142), or against anaphylactic shock (86) has also been denied

3 *Changes in environmental temperature* Either an appreciable increase (491) or a decrease (166, 169, 491, 90, 519, 200, 201) in the external temperature

will precipitate a fatal collapse in the adrenalectomized animal The physiological reactions of the subject to cold have been carefully studied (200, 201) and a bioassay constructed on the basis of the results (405) The primary deficiency is an inability of the adrenalectomized animal to increase its basal heat production, attributed both to a general muscular asthenia and to a circulatory deficiency The body temperature falls rapidly, and death follows Unilaterally adrenalectomized animals also show a decreased tolerance to exposure to cold (201) Since adreno-cortical transplants offer complete protection, a deficiency of the adrenal medulla is apparently not involved (519) Restoration of the resistance to that of the normal animal can be obtained with cortical extract, and with all the cortical hormones, including desoxycorticosterone (405, 234, 523)

4 *Changes in environmental pressure* The suggested relation of the adrenal cortex to the ability of an animal to become acclimatized to a reduction in barometric pressure is based on several points of evidence First, the adrenalectomized animal shows little or no capacity for adaptation, so that moderate pressure reductions are fatal The power of adaptation can be restored by cortical extract or by the corticosterones (253, 265, 456, 475) Second, the intact animal shows a marked hypertrophy of the adrenals after subjection to lowered pressure (456, 475) Third, adaptation is accompanied by a rise in liver glycogen levels and a rise in urinary nitrogen excretion The increase in carbohydrate stores is in large part accounted for by the catabolism of endogenous protein (94, 253, 265) Fourth, the symptoms observed during the post adaptive stage resemble in almost all respects those typical of adrenal insufficiency (456) The interpretation advanced is that the first subjection to lowered pressure increases the demand for the secretion of the adrenal cortex, so that a period of partial adrenal insufficiency follows, corrected only when the glands have become hyperfunctional The eventual failure of the compensating mechanism possibly coincides with exhaustion of the adrenal cortex, and the development of an acute adrenal insufficiency

In the light of this interpretation, it would be reasonable to assume that cortical extract would have a marked effect in allowing the normal animal to survive low pressures. Sundstroem and Michaels (456) give evidence in support of this suggestion, but the results of other workers are not so convincing While cortical extract will correct the loss of sodium and chloride through the kidney (265) its effect in protecting life is not marked (265) Hence the evidence does not yet warrant the conclusion that the adrenal cortex is the sole or even a major factor concerned in adaptation to low pressures

5 *Traumatic injuries* The clinical picture and the blood chemistry changes shown by the animal in adrenal insufficiency are closely similar to those which characterize shock in the normal animal suggesting that a common physiological deficiency might be involved (448) Loss of the adrenals does render the animal exceedingly sensitive to but minor amounts of trauma (437, 111, 451, 333, 332, 444, 490, 193, 402) Swingle and associates showed that none of the major features of adrenal insufficiency, such as hemoconcentration and internal fluid readjustments, changes in serum electrolyte concentrations, or the rise in blood

urea nitrogen levels, are essential characteristics of the shock-like circulatory collapse which follows trauma in the adrenalectomized dog (444, 355) A typical state of adrenal insufficiency has not, therefore, merely been precipitated by the experimental procedures

It was shown earlier (449, 448), however, *a*, that the one most constant symptom of adrenal insufficiency in the dog was a slowly declining blood pressure, *b*, that the arterial pressure started to decline slowly but progressively from the time maintenance amounts of cortical extract were withdrawn, *c*, that the pressure could not be spontaneously raised to normal once it had fallen, and *d*, that the terminal collapse of the animal was always associated with extremely low blood pressure levels The circulatory failure was attributed to a collapse of the peripheral circulation

That the adrenal cortex is concerned in the ability of the normal animal to withstand traumatic injury would seem not unlikely, and has been postulated (193, 402) The proof would lie in the clear demonstration of a protective influence of cortical extract against the development of shock in the intact animal To date such proof has not been forthcoming

The use of cortical extract with saline or plasma transfusions was reported beneficial in treatment of toxemic shock following intestinal obstruction (511) and the injection of the contents of a closed intestinal loop (198) Fine and Fuchs and Mark (105) found that while desoxycorticosterone could prevent the decline in plasma volume after intestinal obstruction, it had no clear effect in preventing shock Selye and associates (401,402) and Weil and co-workers (489) likewise reported that desoxycorticosterone was of no value in treating shock in rats and rabbits following intestinal trauma, but found that cortical extract or the corticosterones were useful Helfrich, Cassels and Cole (195) observed that the blood pressure decline following intestinal manipulation was retarded by cortical extract therapy

In the treatment of shock following hemorrhage, Helfrich and associates (195) reported a favorable effect of cortical extract, but this has been denied by both Fine, Fischmann and Frank (104) and Huizenga, Brofmann and Wiggers (206) in controlled experiments

Katz and co-workers (229) and Shleser and Asher (409) presented rather striking evidence that both desoxycorticosterone and cortical extract could prevent shock in intact dogs subjected to an occlusion of the veins of one hind leg, but their results were not confirmed (436) Bourque and associates (27) found that while cortical extract was ineffective in preventing shock after venous occlusion, it did increase the efficacy of a salt infusion

Noble and Collip (327) observed a slight protective action of cortical extract and desoxycorticosterone against shock induced by revolving rats in a specially designed drum No evidence was found for a therapeutic value of either cortical extract or desoxycorticosterone against shock induced in the intact dog by leg muscle trauma, or following release of tight leg tourniquets, by Swingle and co-workers (436) Likewise, Ingle (210) observed no difference in the survival

of rats subjected to leg constriction by tourniquets after treatment with cortical extract, desoxycorticosterone or the corticosterones

Perhaps the best evidence in favor of a beneficial effect of cortical hormones on shock is in the case of severe burns, where plasma electrolyte changes seem to be prevented (504), and the efficacy of a plasma transfusion is increased (358) While considerable work has been done on the use of cortical extract and desoxycorticosterone in treatment of shock in the human, the reports are conflicting and the results difficult to assess (22, 222, 231, 245, 393, 504) It is apparent that in very few instances has the degree of protection reported against shock been of sufficient magnitude to warrant the conclusion that the stress procedure had induced circulatory failure by reason of a sudden partial adrenal insufficiency

Selye (396, 397) has shown a close similarity between the symptoms of adrenal insufficiency and those exhibited by the intact animal when subjected to any of a wide variety of damaging agents, i e , the alarm reaction This, and other lines of evidence, indicates that a partial state of adrenal insufficiency may be involved The basic mechanism involved is believed to be an inability to inactivate, in a normal manner, a histamine-like material released into the blood after subjection to the noxious stimulus Both blood (503) and tissue (369, 370) histamine levels are elevated after adrenalectomy, presumably because histamine is inactivated less readily in the adrenalectomized animal (368)

In a study of the protection afforded by the various cortical steroids against circulatory failure in the adrenalectomized dog, Swingle and associates (435, 452, 243, 331, 355) found a correlation between the ability to prevent fatal circulatory collapse and *the influence exerted upon carbohydrate metabolism.* They concluded that the most likely interpretation is that the peripheral vascular system of the adrenalectomized animal had become weakened through a failure in intermediary metabolism, so that it was no longer able to maintain normal function in the face of a prolonged stimulation Since there is no evidence of a primary failure of tissue metabolism in shocked intact dogs, it is not surprising to find that cortical hormones are without marked effect in increasing the normal resistance to shock.

On the other hand evidence has been presented that functional deficiencies of the heart, the arterioles and the capillaries may all be involved in the circulatory failure of the adrenalectomized animal suffering from adrenal insufficiency or shocked by traumatic procedures

G *Heart, arterioles and capillaries in adrenal insufficiency* 1 *Heart* Irregular heart rhythms are more or less frequent in acute adrenal insufficiency Nicholson and Soffer (324) have suggested that they may be attributed to the rise in serum potassium concentrations Cleghorn and associates (54, 55, 110) believe that a part of the circulatory collapse can be attributed to cardiac failure Both cortical extract and desoxycorticosterone will correct the cardiac abnormality (55)

2 *Arterioles* The evidence in support of an impairment of the functional

capacity of the arterioles in the adrenalectomized animal is entirely indirect Hypertension cannot be produced in the adrenalectomized animals, even though salt is given (133, 25, 329, 63, 99) The sensitivity to injected renin is also lost shortly after the withdrawing of cortical extract (502, 115, 354), but can be restored by either cortical extract or desoxycorticosterone

Elliott (89) first pointed out that splanchnic stimulation did not evoke a measurable rise in blood pressure in animals prostrate from adrenal insufficiency Likewise, injected barium chloride and pitressin exert little effect (89, 10, 354) Adrenalin, however, produces a full pressor response (89, 10) This is true only for large doses of adrenalin, and comparable results are obtainable with large doses of renin (354) Coombs (64) and Langsdorf (254) presented evidence that there was a decreased sympathetic tone in adrenalectomized animals Secker (395) believed that this loss in tone was due to a decreased production of the sympathetic chemical mediator, but the evidence upon which this conclusion was based has been contradicted (10, 190, 54) Fowler and Cleghorn (110) found, in acute adrenal insufficiency, no failure of splanchnic constriction and concluded that the absence of pressure rise is to be attributed to a failure of the heart to respond to the increased peripheral resistance Whether cardiac failure can explain the decreased sensitivity to all pressor agents remains to be shown

Wyman and tum Suden (518) found that removal of the adrenals changed the acute response to lethal doses of adrenalin from a rapid pressure fall, accompanied by lung edema, to a slow progressive circulatory failure, which might be taken as evidence for a failure in the capacity of vasoconstriction Another line of evidence implicating the arterioles is derived from a study of the response of the adrenalectomized dog to a graded hemorrhage (355)

3 *Capillaries* Considerable evidence, both direct and indirect, indicates that the capillaries of the adrenalectomized animal in insufficiency are atonic, dilated, and abnormally permeable Such an explanation has been used to explain the fact that in adrenal insufficiency, and in experimentally induced rapid dehydration, the blood volume is reduced far lower than might be expected if there were a free transfer of fluid from the interstial reservoirs (352, 444) A serum transfusion will precipitate a circulatory collapse in the adrenalectomized dog, accompanied by an excessive edema (438) Either cortical extract or desoxycorticosterone prevents the edema formation and maintains life

Menkin (302) showed that the leakage of trypan blue into extravascular tissues after the injection of leukotaxin could be reduced by either cortical extract or desoxycorticosterone Fine and Fischmann (103) confirmed this finding for desoxycorticosterone, but do not believe a change in capillary permeability is necessarily involved Freed and Linder (113) found that while cortical extract and corticosterone would prevent dye leakage after leukotaxin or peptone injections, desoxycorticosterone was without positive effect Menkin (303) has criticized this work on the basis of the experimental technique employed Shleser and Freed (410) found that cortical extract retarded capillary dye leakage after peptone injections, but the corticosterones were ineffective Hechter and associates (192) found that desoxycorticosterone acted like the sex steroids in

increasing capillary permeability in the uterus, but observed no effect in other tissues of the body Hechter (191) does not believe that an abnormal increase in the permeability of the capillaries of the adrenalectomized animal can explain the lack of resistance to histamine injections

Cope and associates (65) observed the protein content of the leg lymph to be almost doubled after adrenalectomy, which they believe evidence for an increased capillary permeability In later experiments Cope (66a) finds that following the injection of radioactive colloid into the blood stream the rise in concentration of radioactivity in peripheral lymph is markedly increased in dogs in adrenal insufficiency as compared to the same dogs in the normal state After administration of cortical extract there is a change in capillary permeability permitting the retention of plasma protein within the blood stream. Levin and co-workers (261) and Hartman and associates (179) report that the long recognized increase in plasma protein in the adrenalectomized animals was due to an increase in the globulin fraction, while the plasma albumin, which is the more permeable fraction, was unchanged or even decreased Levin (260) attributes this to a failure in albumin metabolism

The adrenalectomized frog shows engorgement of the capillaries with stasis of the blood, which can be corrected in some degree with cortical extract (289, 459) Since life cannot be maintained with either cortical extract or salt in these forms, there is a question as to whether adrenal insufficiency in the frog is comparable to that of the higher forms Zweifach and Chambers (524) found that cortical extract would prevent the capillary hyperemia which follows exposure, histamine injections, or manipulations with needles Hymen and Chambers (207) also noted an effect in reducing the edema formation in the perfused frog leg

H *Adrenal cortex in lactation* Considerable work, which cannot all be covered here, has been done on the relation of the adrenal cortex to lactation In general, it is agreed that lactation is not induced in the hypophysectomized animal treated with lactogen unless cortical extract or the corticosterones are given (318, 322, 319, 320, 321, 135) Desoxycorticosterone is ineffective (322) Hartman and his colleagues believe that the adrenal cortex contains a specific lactation hormone, *cortilactin* (420) Others believe that lactation can be influenced by any agent which improves the general health of the adrenalectomized animal, such as sodium chloride (127, 321) and that normal lactation is induced with large amounts of cortical extract (127, 120, 322) or the corticosterones (120)

## SUMMARY

Any attempt to prepare a concise summary of the rôle played by the adrenal cortex in physiological processes on the basis of existing evidence involves grave risk of oversimplification It is tempting, however, to regard two general functions of the gland as pre-eminent, each regulated by a distinct type of steroid hormone elaborated by the cortex The desoxycorticosterones, i e , those lacking a keto or hydroxy group at Carbon 11 appear to be chiefly concerned with the regulation of electrolyte and fluid balance, acting 1, directly upon the renal

tubules to allow them to conserve sodium and water and to release potassium, 2, in a manner less clearly defined, on fluid and perhaps electrolyte partitioning across cell membranes, capillary endothelium and intestinal mucosa, and finally, 3, through their regulation of phases of mineral metabolism, primarily those involving sodium and potassium, to produce secondary effects upon extra- and intracellular hydration. Probably as a corollary of this last effect, the desoxycorticosterones allow the excretion in normal amounts of nitrogenous waste products and other metabolites.

The second established major function of the cortex is concerned with the intermediary metabolism of protein and carbohydrate. The oxygenated $C_{11}$ steroids of the corticosterone type are those primarily involved in these phases of organic metabolism. A fault in the energy metabolism of the tissues of adrenalectomized animals lead to a (for lack of a more suitable term) general "asthenia", presumably, although this has not been demonstrated, of all the tissues, organs and organ systems, which is reflected in 1, failure of the work capacity of the skeletal muscle, 2, decrease in certain renal functions (e.g., elimination of excess water), 3, failure of lactation, 4, notably decreased ability of the vasculature to withstand even minor degrees of stress, 5, increased sensitivity of the organism to certain drugs and toxins.

It should be emphasized that all known facts of adrenal cortex function do not conform to this somewhat arbitrary division of functions of the desoxycorticosterones and corticosterones. The physiological activity of the two types of adrenal steroids overlap in many instances. The corticosterones are by no means entirely devoid of activity in so far as renal function and electrolyte metabolism are concerned, nor are the desoxycorticosterones entirely without effect upon organic metabolism and resistance to stress.

Interpretation of the functional significance of the desoxycorticosterones when contrasted with that of the corticosterones is rendered difficult by the fact that so much physiological data have been obtained with use of the synthetic desoxycorticosterone, which apparently is not elaborated in significant amounts in the gland itself, and which in large doses induces striking effects which cannot be produced by even excessive doses of the most potent natural extracts of the cortex. Among the twenty-odd steroids which have so far been isolated from the cortex, six are definitely known to be capable of maintaining life of adrenalectomized animals. Of the remainder a few may be weakly active, whereas most are devoid of known physiological function. However, it is not improbable that some members of this surprising array of steroid derivatives of the cortex may exhibit synergism when intermingled, as in whole gland extract or the natural secretion of the gland itself, and produce effects in adrenalectomized animals which the isolated steroids are incapable of inducing singly. Moreover, before the rôle of the adrenal cortex in physiological processes can be fully elucidated, much more needs to be known regarding the chemical nature and physiological activity of the amorphous fraction remaining after separation of the known cortical steroids from extract concentrates. While the amorphous fraction is known to exert striking effects upon certain aspects of renal function and to be

very potent in so far as life maintenance is concerned, little else is known concerning it.

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# LIPOTROPIC FACTORS

E W McHENRY AND JEAN M PATTERSON

*School of Hygiene, University of Toronto*

The term "lipotropic" was first used in 1935 by Best, Huntsman and Ridout (1) to describe the action of choline in the prevention and cure of fatty livers For the purpose of this review a lipotropic factor is defined as a substance which prevents or removes an accumulation of excess fat in the liver Three such factors will be considered (I) choline, betaine and other related compounds, and choline precursors such as methionine, (II) lipocaic, the name used by Dragstedt to describe material extracted from pancreas, and (III) inositol

I CHOLINE, CHOLINE PRECURSORS AND RELATED COMPOUNDS The discovery of the lipotropic action of choline was derived from studies on the production and prevention of fatty livers in depancreatized dogs maintained with insulin In 1924 Fisher (2) reported the production of fatty livers in such animals and a similar observation was made simultaneously and independently by Allan, Bowie, Macleod and Robinson (3) Hershey (4), working in the laboratory of J J R Macleod, reported that lecithin could be substituted for raw pancreas as a preventive measure against fatty livers in the diets of the dogs More extended observations on the effects of lecithin were provided by Hershey and Soskin (5), and Best, Hershey and Huntsman (6) showed that choline could replace lecithin in the experimental diet

It is interesting to consider the reason advanced by Hershey (4) for the attempt to use lecithin in places of pancreas The latter had been incorporated in the diet of depancreatized dogs because it was assumed that the removal of the pancreas had caused a deficiency of digestive enzymes which would interfere with the absorption of foodstuffs Hershey reasoned that the development of fatty livers did not result from any disturbance in digestion, but was caused by a derangement of fat metabolism in the liver Reverting to the theory advanced by Leathes and Raper (7) that phospholipids were involved in fat transport from the liver, Hershey decided to feed a phospholipid to the depancreatized animals to supply an essential factor for fat metabolism and transport This explanation was clearly given in Hershey's first report (4) and it is desirable to point out the soundness of his reasoning, the correctness of his hypothesis has been amply verified

In a recent paper Engel (8) has stated that the first observation on the lipotropic action of choline was made by Junkersdorf and Kohl (9) some years previous to the work of Hershey Careful reading of the paper published by these investigators has failed to show any reason for concluding that this is the first report on the lipotropic action of choline Junkersdorf and Kohl studied, particularly, the effects of epinephrine and an epinephrine antagonist, choline, upon the amount of glycogen in the liver Fat determinations were made but were meagre and inconclusive The entire emphasis was upon the pharmacologic

effects of epinephrine and choline. The investigations of Hershey and the subsequent ones by Best and collaborators were approached from an entirely different viewpoint, one which is consonant with all subsequent work on the lipotropism of choline.

The production of fatty livers in depancreatized dogs requires a great deal of time and is expensive, the number of observations which may be made is limited. To overcome these difficulties, Best, Hershey and Huntsman (6) used normal rats in which fatty livers were produced by feeding a diet high in saturated fats. They found that the inclusion of lecithin in the diet prevented the accumulation of fat in the liver. In the light of more recent experiments it might be considered odd that fatty livers were actually secured in the early work. The basal diet, consisting of wheat, oats, corn meal, beef fat and bone-meal, had a choline content of about 100 mgm. per cent. Assuming that the animals ate 10 grams of this diet per day, they thus received 10 mgm. of choline, an amount sufficient to prevent fatty livers of the thiamin type. It would seem possible that the grain in this diet supplied an assortment of the B vitamins which would cause the production of a fatty liver somewhat resistant to the action of choline, but which could be prevented by large amounts of choline, supplied in lecithin, plus inositol, furnished by the grain.

Following the demonstrations that lecithin would prevent fatty livers in depancreatized dogs and in normal rats, Best, Hershey and Huntsman (10) studied the effects of components of lecithin. Sodium oleate, sodium glycerophosphate and ethanolamine were found to be without effect, choline was definitely lipotropic. It was also reported that betaine was active. The field was thus opened for the subsequent extensive investigations on choline and other lipotropic factors.

In the eight years following the initial paper on the lipotropism of choline Best and associates studied a number of aspects of the problem and similar work was begun in other laboratories. After collaborative work in Toronto, Channon began similar investigations in Liverpool. The principal developments will be summarized briefly and some of them considered in detail presently. As in the case of rats, mice were found to develop fatty livers which could be prevented by choline (Best, Huntsman and Solandt, 11). Because the basal diets originally used contained large amounts of choline, it was recognized that diets should be planned to contain as little choline as possible (Best and Channon, 12). The fatty livers produced by the ingestion of cholesterol were studied and it was found that large doses of choline were necessary to prevent such fatty livers (Best, Channon and Ridout, 13; Channon and Wilkinson, 14; Aylward, Channon and Wilkinson, 15; Best and Ridout, 16). Choline had no marked effect in preventing the accumulation of cholesteryl esters in the livers, it did prevent an accumulation of glycerides and, in curative experiments, caused a rapid decrease in glycerides and a slower diminution of cholesteryl esters. In the livers made fatty by feeding high-fat diets or in those caused by the inclusion of cholesterol in the diet, the principal effect of choline appeared to be on the glyceride fraction. Choline did not inhibit the deposition of fat in the livers when rats were poisoned

with phosphorus, but did accelerate the removal of fat from the liver during a recovery period, similar observations were made in rats treated with carbon tetrachloride (Best, MacLean and Ridout, 17, Barrett, Best and Ridout, 18, Barrett, Best, MacLean and Ridout, 19) While most of the results were obtained with high-fat diets it was noted that high-carbohydrate diets caused fatty livers when fed without choline (Best and Huntsman, 20) The deposition of liver fat was found to be affected by the amount of casein in the diet (Channon and Wilkinson, 21, Best, Huntsman and Ridout, 1, Beeston, Channon and Wilkinson, 22, Best, Grant and Ridout, 23) Other proteins, such as gelatin and defatted beef muscle, were found to be much less lipotropic This protein effect was largely explained by Tucker and Eckstein (24), who showed that methionine was lipotropic and the action of methionine was made clear by the demonstration by du Vigneaud and associates (25) that this amino acid provided methyl groups for the synthesis of choline *in vivo* Compounds related to choline were studied (Best and Huntsman, 26, Channon and Smith, 27) Betaine and triethylcholine, among others, were found to be lipotropic Griffith and associates (28) contributed important observations on renal damage and on degenerative changes in other tissues, produced in rats fed a diet free of choline, or of choline precursors Further information on the synthesis of choline, indicating the importance of ethanolamine, was provided by Stetten (29) The nature of the mechanism by which choline exerts its lipotropic effect was indicated by Welch (30) and by Perlman and Chaikoff (31) McHenry and associates (32), Engel (8), Forbes (33) and others have investigated the relation of the B vitamins to the production of fatty livers Two other lipotropic factors, lipocaic and inositol, have been described Developing from the initial work on diabetic animals there was, thus, a rapid extension of the investigations on lipotropic factors The results have provided a new approach to the study of fat metabolism and have given new significance to several dietary constituents A more detailed consideration of some aspects of the investigations will now be discussed

1 *The action of choline on various types of fatty livers* In the initial studies on the action of choline in rats, fatty livers were produced by feeding a diet rich in fat The investigations were quickly broadened to include work on the effects of choline on fatty livers produced by feeding cholesterol, by toxic substances, and by other means

*a "Cholesterol" fatty livers* Observations cited by several early workers (34–38) showed that the liver lipids can be changed in nature or in amount by the addition of cholesterol per se or of food substances rich in cholesterol to the diet of experimental animals A more recent study was made in 1931 by Kimura (39) who noted that fat accumulated in the livers of rats which were fed cholesterol Preliminary reports by Blatherwick and associates (40–42) presented evidence which showed that the ingestion of dried whole liver, raw liver, or cooked whole eggs caused fatty livers in rats The fatty liver thus produced was resistant to the lipotropic action of lecithin In later work, Blatherwick et al (43) suggested that one substance, present in these dietary supplements and partially responsible for the observed deposition of fat, might be cholesterol,

although they felt that another factor was present This explanation, in so far as cholesterol was concerned, was accepted by Beeston and Wilkinson (44) Okey (45, 46) in 1933 reported that when cholesterol was fed to white rats as 1 per cent of the diet there was a marked increase in liver fatty acids and in cholesteryl esters but not in free cholesterol That the concentration of free cholesterol in the liver did increase if cholesterol feeding were continued for as long as three weeks was shown by Chanutin and Ludewig (47)

This type of fatty liver, characterized by a high content of cholesteryl esters, has been referred to by Channon and Best as the "cholesterol" fatty liver to distinguish it from the "fat" fatty liver produced by feeding high fat diets It was reported by Best and Ridout (16, 48) that the cholesterol type of fatty liver could be partially but not completely prevented by a quantity of choline sufficient to control the fatty changes produced by the high fat diet alone Excessively large quantities of choline did, however, exert a clearly marked influence on the liver fat In a later paper Best, Channon and Ridout (13) stated that while choline will prevent the accumulation of neutral fat it has no effect on the free sterol and prevents only 60 per cent of the marked rise in cholesteryl ester which results from cholesterol feeding Similar observations, which confirm these findings, were made in the same year by Channon and Wilkinson (14) The importance of the duration of the test period was indicated by the work of Best and Ridout (49), which showed that while choline caused no change in the cholesteryl esters during a twelve-day period it had caused a significant drop in the cholesteryl esters by the forty first day

Aylward, Channon and Wilkinson (15) found that the phospholipid content of the liver decreases for seven hours after a meal containing fat and cholesterol This decrease could be prevented by the administration of choline They suggested that either choline prevented the partial disappearance of liver phospholipid or its presence in the diet permitted the formation of new phospholipid to replace that which had disappeared Further and more extensive observations that the ingestion of cholesterol decreased the liver content of newly formed phospholipid even before the liver was fatty and that choline stimulated the depressed phospholipid metabolism found under these conditions, were made in 1939 by Perlman and Chaikoff (50, 51) Several workers (21, 22, 52, 53) showed that protein exerted a lipotropic action on the glyceride fraction of the liver of rats fed cholesterol It has been pointed out by Best and Channon (12) that the ease of removal and the degree of prevention of liver glyceride deposition in "cholesterol" fatty livers depends in part on the initial glyceride level Best and Ridout (16) have observed that *a*, choline causes a rapid decrease in glyceride and a slower fall in cholesteryl esters, and *b*, that larger doses of choline are necessary to affect the cholesteryl esters than to reduce the glyceride fraction They conclude, therefore, that the primary effect of choline is upon the glyceride fraction, although the possibility that choline affects both cholesteryl esters and glycerides directly cannot be eliminated in view of the fact that cholesteryl esters can be diminished while a large amount of glycerides is still present Experiments by Channon and Wilkinson (14) and Lowndes (54) have shown that the

cholesteryl ester content of the liver is increased by increasing the percentage of fat in the diet The cholesterol fatty liver contains more glyceride than the "fat" fatty liver at any given level of dietary fat

The work which has been reviewed above showed that choline, if given in large quantities, prevented an increase in simple glycerides in the livers of animals to which cholesterol was fed but had comparatively little effect upon the cholesterol content Subsequent investigations have indicated that two other lipotropic agents, lipocaic and inositol, definitely prevent an increase in liver cholesterol These will be discussed in later sections

*b Fatty liver of starvation* During starvation, fat mobilized from the body depots accumulates in the liver (18) A paper on the pharmacological action of choline in starvation, by Junkersdorf and Kohl (9), indirectly called attention to an observation that the fatty infiltration of the liver which occurred on the eleventh day of starvation in dogs receded following choline administration However, the data regarding fat contents were not sufficiently convincing as to warrant a conclusion that choline would prevent the fatty liver of starvation in dogs Best (55) reported that choline administered to rats during a starvation period had little effect on the level of liver fat However, the lipotropic activity of choline was clearly demonstrated if it was administered previous to and during the period of starvation Best and Ridout (56) pointed out that the rat is not a suitable animal for the production of fatty livers by starvation since only adult female rats are so affected Choline does not prevent the fatty liver caused by starvation in the mouse, rabbit or guinea pig Deuel et al (57) investigated the excretion of ketone bodies in the urine of rats previously fed a high fat diet with and without choline and the effect of choline administration to such animals during subsequent fasting They found that choline added to a high fat diet controlled the liver fat (4 27 per cent) and that, during a subsequent period of fasting, liver fat increased (7 83 per cent) The administration of choline during fasting kept the fat at a minimum value and decreased ketonuria It was concluded that choline does not increase the rate of fat oxidation, if ketonuria during fasting is an indication of such oxidation

*c Fatty liver produced by toxic substances* The experimental data presented by Best, MacLean and Ridout (17) showed that choline inhibited neither the deposition of fat nor the production of degenerative changes in the livers of rats following the injection of large amounts of phosphorus Choline did, however, increase the rate of disappearance of fat from the liver during the recovery phase This latter observation was made also in 1936 by Laszt and Verzar (58) Large doses of choline prevented the accumulation of fat in the liver in rats poisoned with carbon tetrachloride, small doses were without effect (19) In investigations with carbon tetrachloride Barrett, Best and Ridout (18) showed by the use of deuterium that the increase of liver fat was due to a withdrawal of fat from the depots

In connection with prevention of fatty livers by toxic agents it should be noted that Sato (59) reported in 1926 that the administration of an extract of liver lowered mortality in rabbits treated with chloroform Subsequently Forbes,

Neale and Scherer (60) studied the preparation and value of a liver extract for the prevention of necrosis in the livers of rats poisoned with carbon tetrachloride vapor The active constituent of the liver extract was claimed by Neale (61) to be xanthine Confirmation of the results with liver extract and with xanthine was provided by Barrett, MacLean and McHenry (62) These workers demonstrated that xanthine protected the liver against necrosis and also accelerated healing following exposure of the animals to the toxic substances Later, Forbes (63) pointed out that, while xanthine was effective, there appeared to be present in the particular liver fraction used by him an unidentified protective agent in addition to xanthine

*d Fatty livers produced by anterior pituitary extract* In 1936 and in 1938 Best and associates (18, 64, 65) studied the effects of "ketogenic" fractions of anterior pituitary extracts in causing a marked increase in liver fat at the expense of body fat, it was found that choline did not prevent the fatty livers but hastened the removal of fat in the recovery phase MacKay and Barnes (66) also reported that the fatty liver produced in rats by this means was not affected by the administration of choline, although choline reduced the accompanying ketonuria The ineffectiveness of choline as a lipotropic substance under these conditions was confirmed in rabbits by Mukerji and Guha (67) Experiments reported by several workers in Dragstedt's laboratory (68) showed that lipocaic would prevent the fatty liver produced by the administration of anterior pituitary extract This finding is contrary to that made previously by MacKay and Barnes (66) Dragstedt and his collaborators were of the opinion that the quantities of pancreatic extract used by MacKay and Barnes could not have been expected to exert definite activity In view of the ineffectiveness of choline the reports regarding lipocaic are interesting and this effect of lipocaic deserves further investigation This would be particularly valuable since the mechanism of production of fatty livers by anterior pituitary extracts resembles the processes involved in the production of fatty livers by starvation and by certain toxic agents In all these cases the increase in liver fat is made possible by a withdrawal of lipids from depot stores It would be most useful to ascertain whether lipocaic will prevent such withdrawals, especially since choline does not

*e Dietary cirrhosis of the liver* Connor (69) considered fatty infiltration of the liver a prerequisite of liver cirrhosis and it has been noted by György and Goldblatt (70) that conditions which produce fatty livers in short term experiments lead to cirrhosis in experiments of longer duration Similar deductions were made by Webster (71), Blumberg and McCollum (72) and by Sebrell et al (73, 74)

In 1939 György and Goldblatt (75) reported that liver injury, in the form of acute diffuse necrosis combined with fat infiltration, could be produced somewhat irregularly in young rats on a diet composed of casein 18 per cent, sucrose 68 per cent, melted butter fat 8 per cent, cod liver oil 2 per cent, salt mixture 4 per cent supplemented with thiamin, riboflavin and pyridoxine Diets containing more fat (50 and 70 per cent) and less protein (casein 10 per cent) had previously been shown to produce cirrhosis of the liver in a series of experiments by Blumberg

and associates (72, 76, 77) György and Goldblatt later (70) demonstrated that cirrhosis could be produced invariably when the casein content of the diet formerly used (75) was reduced to 10 per cent These authors therefore considered that the dietary prevention of liver cirrhosis is related to the lipotropic action of casein The incidence and severity of the liver injury were reduced but not completely prevented by a daily supplement of 10 to 20 mgm choline per rat Cystine, in doses of 10 to 50 mgm daily, was shown to accentuate the cirrhosis while choline or yeast inhibited its production The injurious effect of cystine on liver tissue had been reported by Curtis and Newburgh in 1927 (78) and at a later date by Earle and Victor (79) These authors (80) drew attention to the fact that the liver lesions were proportional to the level of cystine in the diet rather than to the total amount ingested and that choline prevented the effects of small doses of cystine but not of large ones Choline in combination with cystine was reported by György and Goldblatt (70) to be more effective than choline alone in reducing the incidence and severity of liver damage Methionine also prevented cirrhosis This action of choline alone or in combination with methionine or cystine was mentioned in the report of Blumberg and McCollum describing experiments in which a high-fat diet low in choline and other lipotropic factors (72) was used

Daft, Sebrell and Lillie (74) produced cirrhosis of the liver in rats when only a small quantity of cystine (0 5 per cent) was added to a diet low in both fat and protein Choline, methionine and casein either singly or in combination prevented the development of cirrhosis In a later paper (81) they showed that choline and methionine exert a preventive action on liver cirrhosis while cystine and methionine have a preventive action on hepatic hemorrhage and necrosis This finding suggested to the authors that cirrhosis is an entity separate and distinct from hemorrhage and necrosis and there is thus offered an explanation for the observation made by the previous workers that choline and cystine in combination were more effective in reducing liver damage than either alone

Fouts (82) has described recently the production in dogs of a syndrome consisting of anorexia, loss of weight, anemia, skin and peptic ulcers, and fatty cirrhotic livers These conditions developed when the animals were maintained for some months on a low-protein ration with thiamin, nicotinic acid, riboflavin, pyridoxine and pantothenic acid Provided that both pyridoxine and pantothenic acid were given, a high-protein diet appeared to protect the dogs Fouts stated that small doses of choline (10 to 20 mgm per kilo) were ineffective, that larger doses (100 mgm per kilo) were partially effective, and that inositol exerted no beneficial effect Partial improvement was secured with large amounts of choline, a powdered liver extract or a "filtrate factor" prepared from rice bran extract After administration of large amounts of choline and powdered liver extract, deficiency signs cleared up rapidly, although hepatic fibrosis was still marked The amount of inositol used by Fouts appeared to be too small to produce an effect and the same comment could be made about the dosage of choline It would be interesting to try the effects of lipocaic in experiments of this kind

This partial review of the literature on dietary production and prevention of liver cirrhosis in experimental animals indicates that this condition is caused by or at least results from, the use of experimental diets which have been shown to cause fatty livers in rats A diet low in protein, methionine, or choline favors the development of cirrhosis Opinions have varied to some extent with regard to the value of choline but, on the whole, it seems to have been beneficial when given in adequate amounts If the type of cirrhosis produced by dietary means in animals is the result of the maintenance of a high level of liver fat for an extended period, another useful approach could be made by the production of "biotin" or "cholesterol" fatty livers While the results secured in animals have been valuable there has, as yet been no proof that any cirrhosis in humans is a consequence of distortions of the diet

2 *Relation of thiamin and other B vitamins to the production of fatty livers and to the action of choline* In 1935 McHenry (83) reported that there appeared to be a complementary effect exerted by thiamin and choline upon the body weight of young rats Animals fed a diet deficient in both choline and thiamin failed to increase in weight after an initial period of two weeks Either choline or thiamin as a single supplement failed to give a sustained increase in weight but this was accomplished when the two supplements were given jointly In a continuation of this work McHenry (84) found that rats would not develop acutely fatty livers on a low-choline diet unless thiamin was provided At levels of fat intake from zero to 58 per cent of the diet thiamin had a marked effect on the amount of liver fat This was true in experiments in which isocaloric feeding was used, the effect of thiamin was more extensive than simply an increase in appetite Since the body fat was increased at the same time as was the liver fat the production of fatty livers was not due to withdrawal of fat from the depots It was also stated that thiamin caused a marked increase in both liver and body fat in animals fed a fat-free high-carbohydrate ration In this case the vitamin seemed to promote synthesis of fat from carbohydrate, a conclusion which was in agreement with a report by Whipple and Church (85) The effect of thiamin in increasing fat by causing synthesis from carbohydrate can be appreciated but no adequate explanation has been offered of the action of the vitamin in producing fatty livers when a diet rich in fat was fed Confirmation of this action of thiamin was furnished by Best and Ridout in 1938 (56)

It was also shown by McHenry (86) that the amounts of thiamin, choline and fat in the diet jointly affect the body weight of young rats If the ration is deficient in thiamin, the inclusion of more than 26 per cent fat prevents a serious loss in weight The effect of fat is augmented by adding choline to the diet and in this case an optimal effect is secured when the diet contains adequate amounts of choline and about 40 per cent fat When thiamin is supplied without choline optimal weight increases are obtained when the dietary fat ranges between 10 and 26 per cent The observations on the effect of fat when included in a thiamin-deficient diet are, of course, similar to those made previously by Evans and Lepkovsky (87) and confirmed in other laboratories since

In a series of reports on the effects of the B vitamins upon fat synthesis,

McHenry and Gavin (88) found that the amount of fat synthesis is augmented by supplying other B vitamins with thiamin, although no synthesis was observed in the absence of thiamin Riboflavin and rice polish concentrate were shown to have an augmentary effect Halliday (89) reported that a deficiency of pyridoxine caused an increase in liver fat, an increase only partially controlled by choline but prevented by a liver extract used as a source of pyridoxine Gavin and McHenry (90) reported that pyridoxine had no effect in lowering liver fat, these workers used pure pyridoxine and it is possible that the liver extract employed by Halliday contained large amounts of choline In 1940 McHenry and Gavin (91) found that the administration of an extract of beef liver to rats, maintained on a fat-free diet, caused acutely fatty livers containing large amounts of cholesterol, provided that thiamin, riboflavin, pyridoxine and pantothenic acid were also supplied Later they showed (92) that biotin, given in place of the liver fraction, had a similar effect and this type of fatty liver was referred to as the "biotin" fatty liver This type is definitely resistant to the action of choline but can be prevented by lipocaic or by inositol The "biotin" fatty liver is not, of course, the first one encountered in experimental work which has been shown to be resistant to choline It can hardly be said that fatty livers produced by feeding cholesterol are readily susceptible to prevention by choline, nor are those fatty livers resulting from a withdrawal of fat from body depots, caused by starvation, by an anterior pituitary extract, or by a toxic agent However, the observations on the "biotin" fatty liver were of value because they clearly indicated that all fatty livers produced by dietary means were not only dissimilar in genesis but also in response to lipotropic agents and probably in lipid composition as well The effects of dietary constituents in altering the response of various fatty livers to lipotropic agents was further emphasized by the work of Engel (8) and of Gavin, Patterson and McHenry (93) In both cases it was shown that the administration of pantothenic acid and of pyridoxine altered the fatty livers so that they were somewhat resistant to the action of choline A different result was obtained by Forbes (94), in this case nicotinic acid was found to increase the amount of cholesterol in the livers

Observations on the effect of thiamin upon the production of fatty livers have been criticized by Griffith and Mulford (95) and by Handler and Bernheim (96) The first group was concerned, primarily, with effects of diet upon kidney lesions To determine whether thiamin, and other B vitamins, had any influence upon the renal damage, the vitamins were added as supplements to a basal diet which contained 6 per cent yeast It would hardly be expected that the extra supply of vitamins would have any effect, under such circumstances, and none was found This is quite different from the results reported by McHenry, who studied the effects of thiamin deficiency Griffith and Mulford found that a restriction in food intake to an amount about two-thirds of that consumed ad libitum protected rats from renal damage From this result they drew the interpretation that thiamin influenced the production of fatty livers by increasing food consumption or growth It should be made clear, however, that McHenry described experiments in which isocaloric feeding was employed and in which thiamin had a definite effect upon liver fat

The experimental data of Handler and Bernheim are in substantial agreement with those of McHenry, and of McHenry and Gavin Deficiencies of thiamin and of other B vitamins tended to prevent increases in liver fat of animals fed a choline-deficient diet Handler and Bernheim studied the effects of various diets upon the rate of regeneration of liver tissue and upon the amount of liver fat in partially hepatectomized rats Thiamin deficiency did not interfere with liver regeneration To one group of 5 animals, which were choline-deficient, very large amounts of nicotinamide were given As the authors had previously reported, the inclusion of 2 per cent nicotinamide in the diet caused a marked loss in body weight The liver weights of unoperated rats and of partially hepatectomized rats were in fair agreement at the end of the experiment, but were not fatty despite the absence of choline from the diet It was concluded that nicotinamide had not interfered with liver regeneration but if the data are examined it is clearly evident that the nicotinamide had so affected the liver as to cause a serious reduction in weight from that found in a comparable group without nicotinamide Griffith and Mulford found that physiologically-sized doses of nicotinic acid had no appreciable affect upon liver fat and it may be suggested that the large doses of nicotinamide used by Handler and Bernheim had produced a toxic effect which had reduced the size and the fat content of the liver Handler and Bernheim drew the following conclusion with regard to the effects of B vitamins "It is suggested that the development of fatty livers in choline deficiency can proceed only when all other dietary factors will permit the growth of the whole rat rather than merely growth of the liver The effect of deficiencies of members of the vitamin B complex in preventing the appearance of fatty livers due to choline deficiency is the result of an impairment of the over all metabolism of the rat rather than some specific defect in the metabolism of the liver " Since this conclusion is based, largely, upon the claim of complete regeneration of the liver in partially hepatectomized rats fed large amounts of nicotinamide, and since the data do not support this inference, it seems wise to conclude that it has not yet been proven that thiamin deficiency does not cause a defect in liver metabolism It may be said, however, that thiamin deficiency does inhibit the production of fatty livers and this is due to an alteration in those metabolic processes in which thiamin exerts an effect

3 *Lipotropic effect of proteins and amino acids* The first observation that protein influences the level of liver fat was indirectly made in 1935 by Best and Huntsman (20) In an attempt to repeat the work of Rosenfeld (97) on the curative effect of sucrose on fatty livers, rats in which fatty livers had developed were transferred to a diet of pure sucrose an 8 per cent increase in liver fat resulted This increase did not take place if the sucrose diet contained 20 per cent casein At the time of this observation they were not certain whether this effect was due to the fraction of a milligram of choline which the diet provided or to a contaminant in the casein or whether casein provided, by its metabolism, betaines in effective amount

While this work provided the first observation of the lipotropic activity of protein, Channon and Wilkinson (21), six months later, were the first to attach significance to the phenomenon Experiments designed to study the effect of

various levels of dietary protein on liver fat showed that the degree of fat infiltration increased from 5 6 to 12 5 per cent as the casein content of the diet was decreased from 50 to 5 per cent The suggestion was made that certain amino acids might be converted into choline or betaine in the tissues Previous to this, Rosenfeld (98) had postulated that choline might be derived from glycine and as early as 1909 Engeland (99) proposed that betaines might arise in the tissues by the methylation of amino acids In 1935, Best and Channon (12) further supported the supposition that some constituent of protein controls liver fat

Beeston, Channon and Wilkinson (22) extended their work to include the influence of casein on the various fractions of liver fat It was observed that an increase in dietary casein from 5 to 30 per cent resulted in a progressive decrease in the amount of liver glyceride and an increase in the amount of lecithin and cholesteryl oleate in the fatty liver produced by feeding cholesterol These results suggested that the lipotropic effect of dietary protein should be differentiated from that of choline In curative experiments Best and Ridout (53) reported that 50 per cent casein reduced cholesteryl esters to normal in rats which had received daily doses of 18 mgm of cholesterol In preventive experiments, Beeston et al (22) were unable to demonstrate a lipotropic effect of casein on cholesteryl esters produced by the feeding of larger quantities (2 per cent of the diet) of cholesterol The striking difference in the quantity of cholesterol used in these experiments may account for such conflicting results

Casein was shown to exert a maximum lipotropic effect when it formed 30 per cent of a diet containing 40 per cent fat One gram of casein was equivalent to 5 to 8 mgm choline in its preventive action on liver fat (23, 100) The choline equivalent of casein was said to be greater in a fat diet than in a sucrose diet

In an investigation of the effects of a number of amino acids, Beeston and Channon (101) added as little as 0 2 per cent cystine to a diet containing 5 per cent casein and 40 per cent fat and found that the quantity of liver fat was doubled The anti-lipotropic effect of cystine could be offset by the inclusion of 30 per cent casein This preliminary study failed to reveal an amino acid with lipotropic activity but it was shown that lysine, glutamic acid, aspartic acid, serine, glycine and phenylalanine had no effect either in increasing or in decreasing the fat in the liver Beeston and Platt (102, 103) found that alanine, arginine aspartic acid, histidine, hydroxyproline, leucine, lysine, proline and valine were inactive, tyrosine showed slight lipotropic activity Singal and Eckstein (104) confirmed the observation that dl-leucine and dl-valine were without effect on liver fat They also reported that djenkolic acid and dl-isoleucine were lipotropically inactive

In the year following the work of Beeston and Channon (101) on cystine, Tucker and Eckstein (24) confirmed the effect of cystine and were the first to report that methionine was lipotropic In the light of this observation, it was suggested that the lower lipid values obtained by Channon and Wilkinson (21), when the diet contained 30 per cent casein, might be ascribed to the methionine content of the protein and that the two sulfur-containing amino acids exert opposite effects on liver fat

The lipotropic effect of methionine was confirmed by Channon, Manifold and Platt (52) on both the glyceride and cholesterol fractions of liver fat. The lipotropic activity of methionine was calculated to be 1/12th that of choline, a figure which was later revised to 1/5th (105). Best and Ridout (106) also confirmed the lipotropic action of methionine and could find no difference between the activity of the d and l isomers in rats. Similar results were secured by Singal and Eckstein (107) in mice on low-protein, high-fat diets. The latter workers proved conclusively that cysteine and homocystine, like cystine, increase liver fat. They also showed that arachin, a protein of low methionine content, did not possess lipotropic activity. The low methionine content of gelatin may explain the lack of lipotropic effect of this protein, mentioned earlier by Best and Ridout (23).

Channon and collaborators (108) reported on the lipotropic activity of a series of proteins and listed them in the following order of decreasing intensity: gromax and whale muscle protein, caseinogen, albumin, beef muscle protein and edestin, fibrin and gliadin, gelatin and zein. In contrast to the results of Best and Ridout (23) they found that gelatin possessed slight but demonstrable lipotropic activity. In reference to this work Tucker and Eckstein (109) pointed out that, in general, the lipotropic activity of the above mentioned proteins paralleled their methionine content as determined by Baernstein (110).

The first suggestion that factors other than the methionine-cystine content of proteins may be involved in an explanation of the lipotropic activity of proteins was made in 1938 by Tucker and Eckstein (109). Contrary to expectation, they found that the addition of 0.5 per cent cystine to a diet of 5 per cent gliadin and 40 per cent fat failed to increase the liver fat values above that produced by the unsupplemented diet alone. A similar failure to demonstrate the cystine effect was observed by Channon et al. (105) when albumin was the source of protein in the basal diet. In view of the fact that both these proteins contain a high percentage of cystine, a more probable explanation may be that these diets provided more cystine than the amount (7 mgm./rat/day) shown by the work of Beeston and Channon (101) to cause a maximum deposition of liver fat. Channon et al. (108) found that albumin, which contains more methionine than [illegible] casein, exerts a slightly less lipotropic effect. In addition, they observed [illegible] various protein supplements behaved differently, depending on the nature [illegible] basal protein. Best and Ridout (106) considered that their findings also [illegible] the hypothesis that methionine could not be the sole lipotropically active [illegible] in protein. They showed that the methionine and cystine content of [illegible] cent casein diet was not as lipotropic as the 30 per cent casein diet [illegible] servations made by Tucker, Treadwell and Eckstein (111) were direct[illegible] Best and Ridout also reported that a limiting lipotropic action of [illegible] was reached at a level of 0.5 per cent. This was confirmed by Chan[illegible] fold and Platt (105) who demonstrated that methionine, added [illegible] to an 8 per cent casein diet exerted an increasing lipotropic eff[illegible] cent, whereas the lipotropic effect of casein increased until it re[illegible] of the diet.

In the light of these findings Channon et al. (105) stated that "since methionine is probably not the only lipotropic constituent of caseinogen, two possibilities arise, either that some other amino acid also exerts a lipotropic action, or alternatively, added methionine is incapable of exerting its full action in the absence of some other protein constituent." They had no evidence bearing on the second possibility although they mentioned a suggestion made earlier by du Vigneaud (112) that the production of ethanolamine (possibly from an amino acid) might be the limiting factor. This was a most interesting speculation since Stetten (113) in the following year demonstrated that ethanolamine acts as a precursor in the biological synthesis of choline.

In view of the divergent findings of Best and Ridout, and Tucker and Eckstein, a reinvestigation of the comparative influence on liver fat values of casein diets and supplements of cystine and methionine in amounts equivalent to those present in casein diets was made by Treadwell, Groothuis and Eckstein in 1942 (114). This study confirmed their previous findings that diets supplemented with methionine were, so far as lipotropic action is concerned, superior to diets containing equivalent amounts of methionine in the form of casein. Treadwell et al. noted that the young rats on the high casein diet ate more food and gained weight more rapidly than rats on the methionine-cystine diet. They suggested that this would involve an increased demand for methionine in the formation of new tissue protein. The validity of this explanation could be determined by the use of the pair feeding technique.

The fact that liver fat values are affected by food intake has been demonstrated by Griffith and Mulford (95) in young rats. They explain the antilipotropic effect of cystine on the basis that 18 per cent casein diets contain an inadequate supply of dietary sulfur and that a supplement of 0.5 per cent cystine raises the metabolic level and thus creates an increased demand for lipotropic factors (115). On the other hand, the work of Treadwell et al. (114) indicates a direct antagonism between methionine and cystine.

Mention should here be made of the important work of du Vigneaud and associates who suggested (112) and later proved (25) that methionine exerts its lipotropic action, indirectly, by supplying methyl groups for the synthesis of choline. This work will be discussed in more detail in the following section.

4. *Biological synthesis of choline.* Since a very extensive review of the work on the bio-synthesis of choline has been presented by du Vigneaud (116) only the salient features will be noted here.

Du Vigneaud et al. (117) showed that homocystine could not support the growth of rats on a basal diet in which amino acids were used as the source of nitrogen and which was supplemented with thiamin chloride, riboflavin, nicotinic acid and ryzamin B. An equivalent amount of methionine was capable of supporting growth under the same experimental conditions. In contrast to the complete failure of growth with homocystine and the vitamin B mixture, the rats were able to grow when tikitiki extract and milk vitamin concentrate were added. It was concluded that there must be some factor present in the tikitiki and milk vitamin concentrate necessary for the methylation of homocystine.

The possibility that choline might be involved was suggested by the observation that fatty livers developed on the original diet but not on the diet supplemented with tikitiki and milk vitamin concentrate (112) Choline was therefore added to the original diet with homocystine The animals gained weight at as rapid a rate as animals which had been given methionine in place of the choline and homocystine The isolation of choline from the tikitiki and milk vitamin concentrate was further proof that it was, at least in part, responsible for the ability of those supplements to enable the rat to utilize homocystine in place of methionine From these results it was inferred that a supply of methyl groups from choline made possible the conversion of homocystine to methionine

Direct proof for the transfer of methyl groups from choline to methionine was demonstrated by du Vigneaud and collaborators (118) in a recent experiment in which deuterocholine and homocystine were fed to rats and deuteromethionine isolated from the tissue proteins Thus the methyl groups of dietary choline are used in the bio-synthesis of methionine Methyl transfer from choline to methionine was shown to occur even in the absence of dietary homocystine or in the presence of dietary methionine The presence in the diet of a methyl acceptor is therefore not a requisite for transmethylation to methionine to take place It was suggested that a precursor of methionine, undoubtedly homocysteine, is formed by the animal during the catabolism of methionine thus enabling methionine to be resynthesized with the methyl group supplied by choline

Direct proof of the reverse transfer of the methyl group from methionine to provide for the formation of choline was later demonstrated by du Vigneaud et al (25) Methionine containing deuterium in the methyl group was fed to rats on a methionine-choline free diet The choline isolated from the tissues contained an amount of deuterium theoretically possible, if it was assumed that all the methyl groups of the choline had come from the deutero-methionine The substrate that is methylated was shown by Stetten (113) to be ethanolamine, which is formed in the body by the reduction of glycine or as suggested by Folch (119) by the decarboxylation of serine Glycine in turn can arise from the demethylation of betaine

Proof that methionine also serves as a source of methyl groups for methylated compounds other than choline in the body and that the various N-methyl compounds synthesized by the animal derive their methyl groups from the "labile" methyl supply of the diet has been presented by du Vigneaud and co-workers (120) who found deuterium in the anserine isolated from rabbit muscle following the feeding of deuteromethionine

Chandler and du Vigneaud (121) have shown that betaine will enable the rat to utilize homocystine in place of methionine although it is less effective than equivalent amounts of choline The action of betaine in this regard is delayed for several days after its administration The authors offer the explanation that choline is the compound which is actually used by the body for the methylation of homocystine but that betaine can be used after the organism acquires the ability to convert sufficient quantities to choline The inequality of activity of

choline and betaine could be accounted for by a less than quantitative transformation of betaine to choline That betaine is not directly converted to choline has been shown by Stetten (29) Betaine containing isotopic nitrogen was fed to rats and it was found that the glycine of the organ proteins and the phospholipid ethanolamine were rich in $N_{15}$ The phospholipid choline contained no more $N_{15}$ than could be accounted for by the conversion of ethanolamine to choline Therefore Stetten concluded that betaine is demethylated to form glycine, little or none of it being directly reduced to choline It would appear that the lipotropic action of betaine is due eventually to synthesis of choline, which is not accomplished directly

Stetten (122) has reported that the conversion of ethanolamine to choline proceeds without hindrance in rats even when the diet is sufficiently poor in labile methyl groups to cause fatty livers Accordingly, the quantity of choline in the body is maintained at a constant level by the biological methylation of ethanolamine This may explain the lack of relation of the choline content of the body to the amount of choline in the diet noted by several workers (13, 22, 123) Handler and Bernheim (124) reported that choline oxidase is greatly reduced or inhibited by the accumulation of fat in the liver and offer this as a possible explanation of the high content of body choline in the absence of available methyl groups On the other hand, in confirmation of Engel's work (125), Stetten and Grail (126) have reported a drop in the choline content of the liver lipids when choline is omitted from the diet They showed that in normal rats 20 to 30 per cent of the lipid nitrogen is choline nitrogen, whereas in animals on a choline deficient diet the corresponding figure is 11 per cent

It has been clearly established that choline may be synthesized *in vivo* provided that methyl groups are available, as from methionine or betaine, and also that there is available a supply of ethanolamine It would not be expected that symptoms of choline deficiency could be produced if the essentials for choline synthesis are supplied in the diet While the validity of this has been established, there has been some confusion in some papers in the literature as to the rôle of methyl groups in fat metabolism Realizing that choline can be synthesized and that the lipotropic action of choline is due to the stimulation of phospholipid formation, it seems unnecessary to assume, as has been done, that methyl groups play a direct part in the lipotropic action Some discussion has taken place regarding the amount of choline present in the tissues of animals which have been maintained on a diet free of choline and of choline precursors On such a regimen rats show a definite increase in liver fat within two days but it has been stated that the quantity of choline in the animal is not reduced The most recent work (Engel, 125, Stetten and Grail, 126, Patterson and McHenry, 127) shows that the phospholipid concentration and the proportion of choline in the liver is decreased, as would be expected

5 *Mode of action of choline* The mode of action of choline as a lipotropic factor has yet to be determined conclusively The most obvious explanation, and one that is supported by considerable experimental evidence, is that choline promotes lecithin synthesis in the liver According to the early explanation of

Leathes and Raper (7), phospholipids are necessary for fat transport from the liver The work of Bloor (128) and that of Sinclair (129) had definitely established the active rôle of phospholipids in fat transport The widespread occurrence of choline-containing phospholipids and the relative constancy in amount with which they occur in tissues (130) has suggested that they are also concerned with cell structure It has previously been pointed out that Hershey began the original investigation on the effects of lecithin in depancreatized dogs with a hypothesis derived from the explanation advanced by Leathes and Raper (7) regarding the transport function of phospholipids In going back over the early literature regarding the lipotropic action of choline it seems strange that this simple concept of the rôle played by choline was disregarded in favor of more complicated and mysterious ones In a space of a few years a series of hypotheses were advanced as, for example, that choline had an unknown effect upon liver function or that it altered carbohydrate metabolism Experimental evidence was not secured to substantiate these theories and the attention of investigators in this field was brought back to the obvious explanation which had formed the basis of Hershey's research

Experimental evidence reported by Welch (30) and later by Stetten (29, 113) shows that dietary choline is used in the direct synthesis of phospholipid In a clear-cut experiment Welch (30) fed rats arsenocholine, which he showed to be lipotropic, and found arsenic present in the lecithin of the tissues Since the methyl groups of arsenocholine are not labile (131) Welch concluded that its lipotropic activity must depend on reactions which involve the intact molecule It is reasonable to conclude that the lipotropic action of choline also involves the intact molecule rather than its labile methyl groups

The methyl groups of choline do not appear to be necessary for its lipotropic activity since triethylcholine and arsenocholine both possess lipotropic activity as demonstrated by Welch but do not contribute to the labile methyl supply as shown by du Vigneaud et al (112, 131) Mawson and Welch (132) have presented evidence which suggests that the hydroxyl group of choline is necessary for its lipotropic action

Stetten (29, 113) fed choline, containing isotopic nitrogen, to rats and found that the choline of the body phospholipids was rapidly replaced by the ingested labelled choline The liver was reported to be the most active organ, with the other thoracic and abdominal organs next, closely followed by the carcass The brain phospholipids are by far the least active with regard to choline turnover

Perlman and Chaikoff (31) used radioactive phosphorus and showed that choline markedly stimulates the synthesis and transfer of phospholipid by the liver of the fat fed rat Choline caused a stimulation of phospholipid activity about one hour after its ingestion and its effect was still demonstrable for about 10 hours It was observed that there was a relation between the amount of choline ingested and the phospholipid response within a range from 0 to 30 mgm This observation is further evidence for the specific action of choline upon phospholipid metabolism

Channon et al (133) assumed that, if the lecithin hypothesis were correct, any

base having a choline-like action on liver fat should be incorporated in a new phospholipid molecule as a substitute for choline Triethylcholine could not be detected in the liver phospholipid, following its administration to rats While this negative result does not support the lecithin hypothesis, it should not be accepted without confirmation On the other hand, Folch and Woolley (134) reported the presence of inositol, which is lipotropic, in certain tissue phospholipids While significance has not been attached to this observation, it supports the theory of the synthesis of phospholipid in response to the administration of a lipotropic factor Also in favor of this hypothesis is the observation made by Aylward, Channon and Wilkinson (15) that cholesterol, which is "anti-lipotropic", causes a fall in liver phospholipid The fall in phospholipid occurred before the liver was fatty and it was checked by the administration of choline They also noted that, in the absence of choline, as the phospholipid diminished, the glyceride increased Welch and Welch (135) stated that the hypothesis that choline chloride is phosphorylated and utilized in the synthesis of lecithin is supported by the finding that the phosphoric acid ester of choline chloride is unaffected by liver phosphatase and therefore protected from oxidation in the liver

Betaine, another lipotropic substance, accelerates phospholipid turnover The magnitude of phospholipid activity was found to vary with the dose of betaine although the increase was not directly proportional to the amount fed (51)

Perlman and associates (136) extended their studies to include the effect of amino acids on phospholipid turnover in the liver and correlated this with lipotropic activity Methionine was shown to produce an elevation in phospholipid turnover Negative results were obtained with glycine, alanine, tyrosine, glutamic acid, asparagine, proline and serine With the exception of tyrosine, which was reported by Beeston and Platt (102) to possess slight lipotropic activity, these findings agree very well with the inability of these amino acids to exert a lipotropic action as previously reported (101, 102, 103)

An exception to the general correlation between lipotropic activity and phospholipid turnover is the finding by Perlman et al (136) that cystine and cysteine, while not possessing lipotropic activity, accelerate phospholipid turnover in the liver Perlman et al, however, point out that the method of administering these amino acids differed from that in which their lipotropic action was studied Observations of phospholipid turnover were made several hours after a single feeding while lipotropic activity was studied several weeks after daily feeding

In the early work of Best and associates (13) on the effect of choline on liver fat it was reported that, while choline increases the percentage of phospholipids in the liver, its administration does not increase the proportion in which phospholipids containing choline occur in the phospholipid mixture As Changus, Chaikoff and Ruben (137) have reported, the total phospholipid content of a tissue bears no relation to its phospholipid turnover Brain, which contains twice as much phospholipid as liver or kidney, has but a fraction of the phospholipid turnover It may be that a certain rate of phospholipid turnover must be maintained in order to prevent fat accumulation in the liver as well as other signs of choline deficiency

Best (55) suggested that choline might affect liver fat by decreasing the amount absorbed or by increasing the oxidation of fat in the liver. Best and collaborators were unable to obtain experimental evidence to support either hypothesis. Deuel et al. (57) reported that choline does not increase the rate of fat oxidation, if ketonuria during fasting is accepted as evidence of such oxidation. MacLean, Ridout and Best (138) confirmed this conclusion by their observation that rats with fatty livers excrete larger amounts of ketone bodies during fasting than normal rats but the extent of ketonuria is not proportional to the excess fat in the liver.

It may be assumed that choline owes its lipotropic activity to its participation in the formation of phospholipids. From the many investigations on choline has come confirmatory evidence for the theory that phospholipids serve to transport fatty acids from the liver. For the formation of at least some of the phospholipids choline is required. This may be supplied in the diet or it may be synthesized *in vivo* if labile methyl groups and ethanolamine are available. In the absence of dietary choline or of choline precursors neutral fats accumulate in the liver. The principal result of the choline investigations has been to show that choline is necessary for the formation of some phospholipids and that fatty livers are produced if the mechanism for the transport of fatty acids is not available.

6. *Other effects of choline.* *a. Renal degeneration.* In 1939 Griffith and Wade (139) presented evidence for a hitherto unrecognized effect of choline deficiency, hemorrhagic degeneration of the kidneys. This deficiency was produced within 10 days in very young rats maintained on a low choline diet and was characterized by fatty liver accompanied by renal enlargement and marked hemorrhagic degeneration of the kidneys, a regression of the thymus and an enlargement of the spleen. Two milligrams of choline per day prevented the degenerative changes in the kidneys while 10 mgm. was required to control the liver fat. It was suggested (140) that choline has a more fundamental rôle than its function in the regulation of the fat content of the liver. The liver was fatty but the kidneys were normal if all the protein of the diet (15 per cent) consisted either of casein or of dried egg white. Hemorrhagic kidneys developed if 0.3 per cent cystine was added or if the protein consisted of 4 per cent fibrin, 8 per cent casein and 3 per cent dried egg white. The renal lesions could be prevented by the addition of choline or methionine. It was concluded (141) that the absolute amount of either methionine or cystine as well as the ratio of the two affects the choline requirement.

Previously Cox, Smythe and Fishback (142) reported the occurrence of hemorrhagic kidneys in rats on a casein diet to which cystine was added and Hartwell (143) noted similar lesions resulting from high edestin diets. The lesions could be prevented by increasing the supplement of marmite. Griffith's work (141, 144) suggests a correlation between these lesions and choline deficiency.

The histological changes in the kidney resulting from choline deficiency have been described by Christensen (145) and by György and Goldblatt (146) who pointed out their similarity to those resulting from cystine intoxication. Sup-

plements of cystine, fat or cholesterol, in the absence of choline, increase the severity of hemorrhagic degeneration of the kidney (147) The aggravating effect of dietary cholesterol is prevented during the crucial 8 day period but not during a subsequent 30 day period (95)

The age, weight and sex of rats affect the development of symptoms of choline deficiency (140, 148) It was found that renal lesions and fatty livers were most readily produced within 7 to 10 days in 40 gram male rats 20 to 30 days old A marked decrease in the incidence of renal degeneration and liver fat content occurs in older and heavier rats This suggests that there is a corresponding decrease in the choline requirement of older rats Engel has reported (149) that symptoms of choline deficiency can be produced within 7 to 14 days at any time during the period of growth

The choline requirement of the rat appears to be related, also, to the level of metabolism It was noted (140, 144) that, if the dietary protein was adequate in amount and in composition for good growth, renal lesions and high levels of liver fat were produced more readily than if the protein was inadequate Renal hemorrhage can be prevented by restricting the intake of a diet which when fed ad libitum causes the lesion (95) In the light of these observations and since the apparent injurious effect of cystine is not proportional to the level of added cystine, Griffith offered a tentative explanation of the injurious effect of cystine added to diets low in choline, methionine and cystine On such diets there is insufficient methionine to satisfy the demand for choline and possibly for cystine also A supplement of cystine, by improving the state of nutrition of the rat, increases the need for methionine and choline (150)

Since the studies of du Vigneaud et al (25) showed that transmethylation occurs in the animal body and that methyl groups in such utilizable forms as methionine, choline and betaine may be essential in the diet, Griffith suggested that hemorrhagic degeneration of the kidneys might result from a deficiency of the indispensable labile methyl supply (150, 151) Methionine or betaine contribute to the labile methyl supply and may be substituted for choline in the prevention of hemorrhagic kidneys

In animals which survive the crucial 10 day period of choline deficiency the kidney lesions and renal function show spontaneous improvement The liver remains fatty throughout the period of recovery of the kidney, the recovered kidneys remain enlarged and in some cases are scarred with a white incrustation (148)

Engel and Salmon (152) used arachin, which contains very little methionine, as a source of protein in the diet of rats and observed fatal hemorrhages in the kidneys, eyes, adrenals and lungs Weichselbaum (153) reported fatal liver hemorrhages in rats on diets deficient in cystine and methionine These studies serve to confirm the importance of dietary choline for the maintenance of cellular nutrition

Griffith (28) pointed out the probability that the choline phospholipids are involved in an explanation of renal hemorrhagic degeneration since lecithin is an intermediate in fat metabolism and important in cellular structure He sug-

gested that "it is also possible that manifestations of choline deficiency, other than the fatty liver, may be due to the failure of formation of other essential methyl-containing metabolites" Previously Griffith and Mulford (151) had postulated that the renal degeneration was due to a lack of labile methyl groups, mainly because the feeding of methionine and betaine would prevent the lesions It has been clearly established that the rat can synthesize choline from a supply of methyl groups in the diet It would seem reasonable that methyl groups are needed merely for the synthesis of choline and not per se for the prevention of hemorrhagic kidneys since Welch found that triethylcholine (154) and arsenocholine (155), neither of which contribute to the labile methyl supply, prevent the development of hemorrhagic kidneys in rats on a low choline diet

Observations made recently by Patterson and McHenry (127) have shown that renal damage, resulting from a low choline diet, is preceded by a decrease in the concentration of phospholipids in both the liver and kidney Triethylcholine or choline prevent kidney damage and maintain the phospholipid concentration within normal levels On the basis of these observations it was suggested that kidney lesions may result from a failure of a sufficiently rapid rate of phospholipid formation to maintain the structure of the growing kidney While this hypothesis has yet to be proven it fits many of the known facts Griffith (151) found that 1 to 2 mgm choline would prevent the renal lesions, while 6 to 8 mgm were necessary to reduce the liver fat In accordance with the stimulating effect of choline on phospholipid turnover noted by Perlman and Chaikoff (31), it is conceivable that a small quantity of choline (1–2 mgm ) would stimulate turnover sufficiently to provide enough for the more vital cell structure while a large amount (6–8 mgm ) would be necessary to exert a demonstrable effect on liver fat Griffith and Wade (140) have stated that renal hemorrhage can be produced most easily in only one brief portion of the rat's life, that immediately after weaning, and Engel (149) noted that it could be produced at any time during the period of rapid growth During growth a supply of phospholipid may be particularly essential for the development of new cell structure Changus, Chaikoff and Ruben (137) have shown that the rate of phospholipid turnover is much greater in younger rats than in older ones Therefore, if choline is necessary to maintain phospholipid turnover, one could account for the requirement of choline being greater in young rats than in mature ones and also for the spontaneous recovery of the kidney at a time when the rate of phospholipid turnover is normally being reduced Methionine, betaine and choline, which prevent renal damage, also stimulate phospholipid turnover It should be noted that all substances which prevent this type of renal damage are lipotropic agents and it seems unnecessary to postulate any causative mechanism other than the rôle played by these substances in phospholipid formation and consequent fat transport

*b Hyperplasia of the forestomach in rats* Hyperplasia and ulceration of the epithelium of the forestomach of rats fed bread and wood shavings was first observed in 1914 by Singer (156) Since that time several investigations have been made to determine the etiology of the lesion While irritation by parasites (157) or by gastric juice (158) has been the only explanation advanced for the

direct production of the lesion, numerous reports have suggested that various dietary deficiencies are a predisposing factor (159, 160, 161, 162)

Dalldorf and Kellogg (163) and Sure and Thatcher (164) reported stomach lesions in rats suffering from thiamin deficiency Similar lesions were obtained by Findlay (165) with a deficiency of vitamin $B_2$ Howes and Vivier (166) demonstrated that gastric lesions occur as a result of a deficiency of the vitamin B complex This observation was confirmed by Sharpless (167, 168) who showed that lesions could be produced by a dietary deficiency either of protein or of the vitamin B complex

Sharpless (168) demonstrated that the epithelial changes can be promoted in most rats fed a diet of white flour by providing supplements of riboflavin, nicotinic acid, cystine and an extract of rice polishings In a later study Sharpless and Sabol (169) substituted thiamin, pyridoxine, choline and calcium pantothenate in various combinations in place of rice polish extract When pyridoxine was added to the white flour diet containing thiamin and riboflavin, no protection was secured, choline protected all but 31 per cent of the animals, pyridoxine and choline fed together reduced the incidence of gastric lesions to 14 per cent, the further addition of calcium pantothenate had no additive effect The results seemed to indicate that pyridoxine in the presence of choline aids in maintaining normal gastric epithelium It was noted that animals fed the basal diet without choline had intestinal contents in the stomach The stomachs of animals fed choline were free from visible intestinal contents Sharpless and Sabol suggested that choline stimulated the smooth muscle of the gastro-intestinal tract and helped to prevent regurgitation of bile which might act as a stimulus to cell proliferation

Sharpless (170) has reported additional data as a basis for an explanation of the gastric lesions He found that sodium taurocholate or pepsin or hydrochloric acid increased the incidence of hyperplasia of the forestomach epithelium in animals fed diets deficient in cystine, riboflavin, pyridoxine or choline but not in animals fed a nutritionally adequate diet The action of the protective factors is interdependent and a deficiency of one may prevent effective action by others Sharpless postulated that "the mechanism of formation of gastric lesions is the irritation of abnormally sensitive epithelium by hair, hard food particles, pepsin and hydrochloric acid, or bile" The relation of choline to the prevention of these lesions is not clear

c *Lactation* Sure (171) has reported that choline is an indispensable component of the diet for growth and lactation of the rat When lactating rats were maintained on a diet deficient in choline the growth of the young suckling rats was arrested by the 13th day and paralysis and death ensued The administration of 15 mgm choline per day to the young rats or 5 mgm choline to the mother rats and 10 mgm to the young rats was required for satisfactory growth of the young Choline was also shown to be necessary for growth of the weaned rat The need for choline by the young rat is probably not a manifestation of a new function of choline but is related to the requirement of choline for normal phospholipid formation which is intimately concerned with fat transfer Suckling

rats are receiving a high fat diet, since maternal milk contains 32 per cent fat (171). The phospholipid turnover is higher in the suckling rat than during any period of growth and consequently there would be a greater demand for choline during this period (172, 173). Artom and Fishman (174) have recently shown that choline administration is necessary to maintain the normal concentration of choline phospholipids in the liver of the newly weaned rat, maintained on a diet containing small amounts of choline or of choline precursors.

(d) *Perosis.* Perosis or "slipped tendon" has been extensively studied in the chick. Two excellent reviews of the studies on the preventive effect of manganese on this syndrome have been presented by Jukes (175) and by Wilgus et al. (176). In this review reference will be made only to the relation of choline to perosis. In 1939 Jukes (177) observed that turkeys were highly susceptible to perosis and that it was not prevented in this species by the inclusion of manganese in the diet. In the following year Jukes (178, 179) described experiments which showed that choline, in addition to manganese, was effective in preventing perosis in both turkeys and chicks. It was noted that an adequate supply of the vitamin B complex was necessary for the full anti perotic effect of choline to be exerted and that arsenocholine was anti-perotic while gelatin was completely ineffective. In contrast to the results secured with turkeys, Jukes (180) observed that chicks did not develop perosis on a choline deficient diet unless gelatin or creatine were added. Choline at a level of 0.1 per cent prevented perosis under these conditions. This effect of creatine was explained on the basis of the observation made by Almquist and Mecchi (181) that creatine deficiency results in muscular dystrophy and, if the tension on the bones is reduced by muscular dystrophy, the tendency toward perosis may be lessened. Glycine, the principal amino acid in gelatin, is a precursor of creatine. A study by Jukes and Welch (182) of the effect of certain analogues of choline on growth and on perosis in chicks showed that the anti perotic activity of choline is distinct from its growth promoting action. That fatty livers are not concerned in perosis resulting from choline deficiency was observed by Hegsted, Mills, Elvehjem and Hart (183), who confirmed the need for choline in the diet of the chick and noted that chicks suffering from perosis due to choline deficiency at 4 weeks of age did not show fatty livers. Hogan et al. (184) agree that choline definitely has perosis-preventing activity but their investigation indicated that choline was not the only specific nutrient present in such natural anti perosis supplements as liver. No adequate explanation has been provided regarding the function of choline in the prevention of perosis.

(e) *Acetylcholine formation.* A preliminary experiment made by Solandt (185) suggested that a dietary deficiency of choline may result in a deficient formation of the neurohumour, acetylcholine. This hypothesis was tested in later experiments (186) in which it was observed that vagus stimulation reduced the heart rate to approximately 30 per cent of the normal value in rats on a normal diet, to 45 per cent in rats on a low choline, plus choline, diet, to 75 per cent in animals on a low choline diet. Intravenous injections of choline chloride in the latter group increased the action of the vagus on the heart muscle. This

phenomenon was never observed in rats in either of the groups receiving choline regularly Solandt and Best concluded that a low intake of dietary choline causes deficient vagus function which can be rectified by injected choline It was suggested that choline deficiency results in deficient formation of acetylcholine at the nerve endings No more definite connection between choline in the diet and the liberation of acetylcholine at the nerve endings has so far been reported These observations are of interest because they indicate that dietary choline may serve in a capacity quite different from that which explains the lipotropic function

(*f*) *Butter yellow, hepatic tumors and choline* The effect of various dietary factors on the hepatic tumors produced by adding butter yellow to a diet of unpolished rice and a small amount of carrot have been investigated by numerous workers

The incidence of tumors in rats was markedly reduced by rice-bran oil (187), yeast (188, 189), dried beef liver (190) or the replacement of rice by wheat bread (188, 191) Sugiura and Rhoads (192, 193) found that an extract of rice-bran or an ether extract of yeast caused a transitory inhibition of liver cancer

Kensler, Sugiura and Rhoads (194) reported that the contents of coenzyme I and of riboflavin in the livers of rats on the basal rice diet plus butter yellow were markedly reduced below normal values The addition of 15 per cent whole yeast increased the levels to normal In the following year these workers (195) studied the effect of nicotinic acid, riboflavin, and casein on the susceptibility of rats to cancer These three supplements administered separately gave no protection but the administration of nicotinic acid with riboflavin resulted in a definite decrease in cancer incidence, while casein plus riboflavin gave striking though not absolute protection They concluded that at least two factors, riboflavin and casein, render the rat less susceptible to the carcinogenic effect of butter yellow Gyorgy, Poling and Goldblatt (196) confirmed the beneficial effect of casein and noted that the combined administration of cystine plus choline afforded definite but not regular protection against the pathological changes in the livers produced by butter yellow Du Vigneaud et al (197) found biotin to be procarcinogenic when administered to rats receiving butter yellow and an otherwise highly protective diet

Miller et al (198) studied the effects of various modifications in the basal ration on the rate of tumor formation with butter yellow, particular emphasis being placed on the relative effectiveness of various proteins Dietary casein (18–40 per cent) offered partial protection, liver, yeast and egg gave equivalent protection at levels of 12 to 13 per cent of total protein, 10 per cent whole dried beef liver in a 10 per cent casein diet or at a 20 per cent level as the sole source of protein offered nearly complete protection at 4 to 6 months, 1 2 to 2 per cent water-soluble, alcohol-insoluble portion of whole liver in a 10 to 12 per cent casein diet gave nearly complete protection at 4 months but not at 6 months, xanthine, l-cystine, i-inositol, and choline (0 1–0 5 per cent levels) gave no protection Feeding experiments indicated that the dye reduced growth per se as well as by causing a decreased food intake The authors concluded "(a) that nutritionally adequate diets offered at least partial protection against hepatoma formation

(b) that protective supplements were usually rich in both protein and the vitamin B complex, particularly riboflavin (c) that non protective diets were deficient in at least one of these factors"

Jacobi and Baumann (199) demonstrated that butter yellow contains labile methyl groups and suggested that it may cause tumor formation by stimulating phospholipid turnover which is higher in tumor tissue than in normal tissue Choline was found to be without effect on tumor growth However, the tumor tissue may draw on the host for its supply of choline or it may itself synthesize this substance.

(7) *Is Choline a Vitamin?* There has been some debate as to whether choline should be classified as a vitamin, and more particularly as a member of the vitamin B complex It is difficult to see why choline should be called a vitamin The word vitamin was originally used to describe an unidentified substance, believed to be an amine, present in foods in minute amounts, and necessary for life The chemical constitution of choline was known before any dietary significance was attached to it No useful scientific purpose would be served by considering choline to be a vitamin The attempt to call choline a B vitamin is even less excusable There is evidence, either conclusive or suggestive, to warrant the opinion that the B vitamins are components of enzyme systems There is no evidence that choline acts in this capacity The only function of choline for which there is definite evidence appears to be that it is a structural constituent, either in the formation of phospholipids or for a supply of methyl groups It would appear to be more satisfactory to leave choline unclassified, other than to say that, under certain conditions, it is an essential constituent of the diet This is particularly true in view of the commercial exploitation of vitamins The abortive attempt to call an essential fatty acid a vitamin is familiar to everyone, as is the effort to use this classification for commercial purposes The reviewers can see no advantage, and several disadvantages, in labelling choline as a vitamin. It should, indeed, be remembered that choline only becomes a dietary requisite when the supplies of methionine and ethanolamine are markedly restricted

(8) *Lipotropic Activity of Compounds Related to Choline* Experiments described by Best and Huntsman (26) to study the effect of various components of lecithin on the fatty liver of the rat showed that betaine has a lipotropic action Platt (200) reported that the activity of betaine, in this regard was about 30 per cent that of choline In an experiment which indicated that the hydroxyl group of choline is necessary for its lipotropic action, Mawson and Welch (132) reported that trimethylammonium chloride caused an increase in liver fat, while trimethylethyl, tetramethyl and trimethylphenylammonium chloride were so toxic that measurements of lipotropic activity could not be made According to Channon and Smith (27) triethyl $\beta$ hydroxyethylammonium hydroxide possessed two-thirds the lipotropic activity of choline weight for weight Tripropyl $\beta$ hydroxylethylammonium hydroxide had little, if any, lipotropic effect while homocholine (trimethyl $\gamma$ hydroxypropylammonium hydroxide) was more effective than choline itself on both the fat fatty liver and the cholesterol fatty liver

(201) Observations by Welch and Welch (135) indicated that N-betaine aldehydechloride, and N-betaine hydrochloride, and the phosphorus and arsenic analogues of choline chloride were more than one-half as active as was choline chloride Phosphorus and arsenic betaine hydrochloride were not lipotropic This suggests that the phosphorus and arsenic analogues of choline act per se, or as corresponding aldehydes Platt (200) has reported that etherification of the alcohol group of choline to form choline methyl ether destroys its lipotropic effect, tetra β hydroxyethylammonium chloride has no lipotropic activity

TABLE 1

*Lipotropic activity of compounds related to choline*

| COMPOUND | LIPOTROPIC ACTIVITY | REFERENCE |
|---|---|---|
| Arsenobetaine hydrochloride | − | 135 |
| Arsenocholine chloride | + | 135, 30 |
| Betaine | + | 26, 135 |
| Betaine aldehyde chloride | + | 135 |
| Betaine aldehyde acetyl chloride | + | 135 |
| Calcium phosphorylcholine chloride | + | 135 |
| Choline chloride | + | 26, 10, 135 |
| Choline methyl ether | − | 200 |
| Diethylmethylhydroxyethylammonium chloride | + | Cited in 131 |
| Dimethylethylhydroxyethylammonium chloride | + | 131 |
| Homocholine | + | 201 |
| α methylbetaine hydrochloride | + | 135 |
| β methylcholine chloride | − | 135 |
| β methylcholine ethyl ether | − | 135 |
| α methyl-β-phenylcholine chloride | ? | 135 |
| Phosphobetaine hydrochloride | − | 135 |
| Phosphocholine chloride | + | 135 |
| Tetra-β-hydroxyethylammonium chloride | − | 200 |
| Tetramethylammonium chloride | toxic | 132 |
| Triethyl-β-hydroxyethylammonium hydroxide | + | 27, 133 |
| Trimethylamine oxide hydrochloride | − | 135 |
| Trimethylammonium chloride | − | 132 |
| Trimethylethylammonium chloride | toxic | 132 |
| Trimethylphenylammonium chloride | toxic | 132 |
| Tripropyl-β-hydroxyethylammonium chloride | − | 201 |

A summary of the qualitative lipotropic activity of various compounds related to choline is given in table 1

From a consideration of the various compounds which have been shown to be directly lipotropic it would appear that the general configuration of the choline molecule is essential but that some particular segments are not Methyl groups may be replaced by ethyl, as in triethylcholine Nitrogen may be replaced by other elements with suitable valency (arsenic, phosphorus) A free hydroxyl group does seem to be necessary These statements apply to compounds which, like choline, directly participate in the formation of phospholipids but not, of

course, to those substances which exert a lipotropic effect indirectly because they supply groupings which are required for the synthesis of choline, or of a choline-like compound

(9) *Distribution of Choline* Several methods have been used for the estimation of choline It is widely distributed in animal tissues, as would be expected in view of the occurrence of lecithin It has not been realized by some investigators that many substances used in the preparation of experimental diets are excellent sources of choline This is particularly true of tissue extracts which have been employed to supply unidentified vitamins. It has seemed useful to summarize available information regarding the occurrence of choline in substances used in animal diets, mainly for the purpose of emphasizing precautions which must be taken in investigations in which effects of choline are to be studied. Practically all of such information is contained in two papers, one by Fletcher, Solandt and Best (202), the other by Engel (203) Table 2 gives the average values recorded by these workers

A number of instances are available in the literature to demonstrate the need for care in planning animal dietaries In the initial investigations by Best and associates (10) a grain diet was employed, this contained such large amounts of choline that it is difficult to understand how fatty livers were secured. A more recent example is contained in a paper by Quackenbush et al (204) These workers noted that the addition of thiamin to the diet failed to cause fatty livers in rats The diet contained 8 per cent autoclaved yeast, which would have supplied sufficient choline to prevent fatty livers If the effects of choline are to be studied, or alterations in fat transport noted, not only must consideration be given to the actual choline content of the basal diet but in addition the presence of choline precursors must be taken into account Choline deficiency cannot be expected unless attention is given to all dietary sources of choline and of those substances which make possible the *in vivo* synthesis of choline

II LIPOCAIC After the initial work by Hershey (4) the assumption could have been derived from the literature that the value of the inclusion of pancreas in the diet of depancreatized dogs was due to its content of lecithin or choline A modification of this assumption was introduced in 1935 by the demonstration of the lipotropic effect of protein Investigations by Ralli, Flaum and Banta (205) in the same year suggested that raw pancreas was more effective in controlling the liver fat of depancreatized dogs than could be accounted for by its content of lecithin

In 1936 Dragstedt and associates began investigations on the control of liver fat in depancreatized animals In their first reports (206, 207) they concluded that the development of fatty livers is not due to the absence of pancreatic juice and that pancreatic tissue contains a lipotropic factor other than choline The authors reported that at least 1 0 gram of choline was necessary per day to prevent fatty livers, while an effective amount of pancreas contained about 60 mgm of choline In view of later developments it should be pointed out that Dragstedt et al tried liver or brain in place of pancreas, not only were these without effect but it might be concluded from the paper that the inclusion of liver in the diet had

accelerated the fatty infiltration of the liver of the experimental animals In extending these observations Dragstedt, Prohaska and Harms described the preparation of an active extract from pancreas The name "lipocaic" was suggested for the component which prevented the development of fatty livers Because it was believed that pancreas alone contained lipocaic it was claimed to be a new hormone concerned with the normal transport and utilization of fat

Significant contributions to the study of the effects of pancreas and choline were made by Kaplan and Chaikoff (208, 209, 210) Unfortunately, one of these observations was lost sight of by subsequent workers and has not yet received adequate attention These workers showed that depancreatized dogs maintained with insulin showed a decrease in blood lipids as well as an increase in liver fat They found that the feeding of raw pancreas prevented the increase in hepatic fat and raised the blood lipids above normal This dual effect was not obtained with choline, or with choline plus autoclaved pancreas They concluded that

TABLE 2

*Choline content of substances used in animal diets*

(Milligrams per 100 grams)

| SUBSTANCE | FLETCHER | ENGEL | SUBSTANCE | FLETCHER | ENGEL |
|---|---|---|---|---|---|
| Beef liver | 270 | | Vitamin free casein | 4 | |
| Beef pancreas | 230 | | Agar | 1 | |
| White flour | 140 | | Crisco | 0 5 | |
| Polished rice | 94 | | Potato starch | 0 | |
| Skim milk powder | 90 | | Cane sugar | 0 | |
| Rice flour | 69 | | Mazola (corn oil) | 0 | |
| Bone meal | 30 | | Dried baker's yeast | 270 | 460 |
| Corn starch | 25 | | Dried brewer's yeast | 240 | 430 |
| Dried egg white | 18 | | Peanut meal | | 225 |
| Rice starch | 10 | | Soy bean meal | | 345 |

pancreatic tissue contains two factors active in lipid metabolism 1, a heat-labile factor which controls blood lipid levels, and 2, a heat-stable substance which prevents fat deposition in the liver The existence of these two particular factors was never subsequently confirmed and their existence might be doubted However, it seems likely that the heat-stable factor was choline, or perhaps choline plus another substance The most significant conclusion derived by Kaplan and Chaikoff, and one which was not considered by most subsequent investigators, was, "although choline influences the fatty livers of rats fed a high-fat, low-protein diet as well as those of depancreatized dogs maintained on a low-fat, high-protein diet, the evidence available at present does not permit the conclusion that the mechanism of production and cure of these two types of fatty livers is the same Hence conclusions derived from experiments on rats cannot be applied at present to depancreatized dogs " If this concept, that all fatty livers are not identical, had been taken into account, some of the subsequent confusion regarding lipocaic would not have appeared

Shortly after the publication of the initial papers by Dragstedt and associates, the lipotropic effect of lipocaic was examined on rats in several laboratories. Overlooking the precautionary conclusion of Kaplan and Chaikoff, all of these investigations except one involved the use of a type of fatty liver which had been shown to respond readily to choline Best and Ridout (211) found that lipocaic had no activity in rats other than that which could be accounted for on the basis of its content of choline and protein While they carefully pointed out that lipocaic might contain a factor affecting the deposition of fat in the liver of the depancreatized dog but not in the "normal" rat, the inference could have been drawn easily that lipocaic had no special effect. Similar conclusions were expressed by Aylward and Holt (212) and by MacKay and Barnes (213) All three investigations were carried out with rats fed a low protein, high fat diet In contrast with these negative findings, Channon, Loach and Tristram (214) reported evidence showing that pancreatic extracts contain a lipotropic agent distinct from choline or protein The positive results were mostly secured in rats which had livers made fatty by feeding cholesterol Unfortunately, in the light of later work, no observations were made upon liver or blood cholesterol values Channon and associates, like Kaplan and Chaikoff, attempted to distinguish between the conventional type of dietary fatty livers in rats and those found in depancreatized dogs They pointed out that depancreatized dogs develop fatty livers on protein rich diets which would inhibit the production of fatty livers in rats

In connection with an investigation of the causes and prevention of fatty livers in depancreatized dogs, Ralli, Rubin and Present (215) noted in 1938 that the livers showed a marked increase in cholesteryl esters but no particular change in free cholesterol In the following year, Dragstedt et al (216) described blood lipid changes co-incident with the development of fatty livers a reduction in total lipids, and of cholesteryl esters. The administration of lipocaic caused an increase in blood lipids

Dragstedt (217) reviewed the status of lipocaic in 1940 and it is advantageous to consider some of the features of the review He stated that the depancreatized dog suffers from two obvious deficiencies insulin and pancreatic juice Adequate dosage with insulin does not prevent the production of fatty liver, nor does a supply of pancreatic juice (see next paragraph) The feeding of raw pancreas is completely effective as a lipotropic agent as also are highly purified pancreatic extracts containing entirely ineffective amounts of choline Under conditions used in Dragstedt's laboratory, the minimal effective dose of choline was found to be 2 grams per day In the review Dragstedt emphasized his belief that lipocaic was an internal secretion of the pancreas If this supposition is granted, the pancreas supplies two hormones insulin and lipocaic After removal of the pancreas the deficiency of insulin is manifest within a few hours as is the case with other hormones, following gland extirpation Fatty livers do not develop for a period of some weeks, or perhaps months According to Dragstedt, these fatty livers are caused by a deficiency of lipocaic How can the very slow development of fatty livers be explained if lipocaic is a hormone? It seems un

and sucrose, that is, a high-protein, high-carbohydrate, low-fat ration, and generally without additional vitamin supplements Dragstedt et al (217) have stressed the large dosage of choline which they found to be necessary and the satisfactory response given by lipocaic Their animals were fed a ration of meat, whole milk and white bread and this contained appreciably more fat than that used by Best and associates With dogs maintained on this diet Dragstedt has failed to find fatty livers after ligation of pancreatic ducts, or that pancreatic juice has a lipotropic effect The basal rations employed by Chaikoff (218) and by Ralli (205) have differed from each other and from those supplied in other laboratories, both Chaikoff and Ralli have included generous amounts of the B vitamins in the diets Great care should be exercised in comparing results between different species of animals but it might well be recalled that the inclusion of a minute amount of biotin in the diet fed to rats causes a marked difference in response to lipotropic agents It is very difficult, if not impossible, to compare the results found in different laboratories in studies on depancreatized dogs in view of the wide variation in the basal rations In the opinion of the present reviewers clarification will not be secured until standardization of technique is adopted, particularly with reference to the basal ration

There are a number of attractive, unsolved questions in the investigations on depancreatized dogs The work of Ralli and Rubin (222), and that by Gavin and McHenry (92) in rats, suggests that there may be a positive agent promoting fat deposition in the liver Is this agent biotin? Biotin causes fatty livers in rats which resemble in several respects at least some of the fatty livers which have been studied in dogs What is the rôle of pancreatic juice? One is inclined to accept the finding of Chaikoff and associates (218–221) and of Ralli et al (215) that the absence of pancreatic juice contributes to the formation of fatty livers Pancreatic juice may supply lipocaic directly or it may cause the liberation and consequent absorption of a lipotropic factor from foods in the basal diet While the contention that lipocaic is a hormone may be doubted, there can be no question that it is lipotropic, nor that this activity is due to a constituent other than choline or protein What is the chemical nature of lipocaic? Very little information is available regarding the active substance The production and prevention of fatty livers in depancreatized dogs still offers fruitful field for investigation

III INOSITOL In 1928 Eastcott (226), working in Lash Miller's laboratory, identified bios I as inactive inositol Woolley (227) showed in 1940 that inositol is an essential dietary factor for mice and that a deficiency of it causes alopecia in that species Deficiency symptoms have also been described in rats by Pavcek and Baum (228) who believed that a lack of inositol causes the so-called "spectacle-eye" syndrome

Gavin and McHenry (225) reported in 1941 that the inclusion of inositol in the diets of white rats would prevent the biotin type of fatty liver, caused by the administration of biotin in conjunction with thiamin, riboflavin, pantothenic acid and pyridoxine Inositol not only prevented the accumulation of fatty acids but also the increase in cholesterol content which is characteristic of this type of fatty

liver It was stated that this lipotropic action of inositol resembled that of lipocaic but it was not said that lipocaic owed its activity to inositol contained in the preparation, although such an inference could have been drawn

Attempts to demonstrate a lipotropic effect of inositol in depancreatized dogs were subsequently reported by two groups of workers (Owens, Allen, Stringer and Dragstedt, 229, Rubin and Ralli, 230) Owens et al found that inositol had some lipotropic effect but not as much as was shown by an identical amount of lipocaic Inositol appeared to have little influence upon prolongation of life, upon insulin tolerance, or upon body fat, lipocaic did affect these It was inferred that, while lipocaic may contain inositol, the various effects produced by it in depancreatized dogs are not given by inositol and that lipocaic probably contains a quite different, unidentified, lipotropic substance A somewhat similar conclusion was drawn by Rubin and Ralli Because of the extensive experience of both groups of investigators one is inclined to accept these results, although it should be pointed out that a small number of dogs was used in both cases and that it was not possible to try a great range of dosage of inositol If the results are accepted, the conclusion is valid that lipocaic contains an unidentified substance It may be added that preparations of lipocaic examined in the reviewers' laboratory have contained sufficient inositol to account for their lipotropic potency in rats

Engel (8) has studied the effects of inositol in preventing fatty livers in rats The animals received thiamin, riboflavin, pyridoxine, pantothenic acid, corn oil and choline in various dosages A maximal effect was obtained with 10 mgm choline per day but this amount did not ensure a normal quantity of liver fat This was secured by providing 3 mgm inositol in addition to choline The lipotropic action of inositol, demonstrated by Gavin and McHenry, was corroborated Although Engel did not use biotin, his results show that a type of fatty liver refractory to choline was secured and that inositol was effective under the conditions used A more recent paper by Gavin, Patterson and McHenry (93) describes several circumstances under which inositol is effective Since this will be discussed in a subsequent section, detailed reference will not be made to it here

Although there is little experimental evidence regarding the mechanism by which inositol exerts a lipotropic action, some interesting speculations may be made Anderson and associates (231, 232) showed that inositol was present in phospholipid combination in the tubercle bacillus and in soy beans Folch and Woolley (134) reported that inositol was contained in considerable amounts in naturally occurring phospholipids of animal tissues It might be suggested that inositol, like choline, owes its lipotropic activity to participation in the formation of certain phospholipids It is of interest that inositol is effective under conditions unfavorable for the action of choline, particularly when large amounts of cholesterol are present in the liver Complete analyses of inositol and choline-containing phospholipids might provide valuable information regarding the mode of action of the two factors It might be possible, for example, that a different selection of fatty acids is made to form inositol phospholipids from those

which combine with choline Inositol would appear to have some relation to cholesterol metabolism but the relationship is unknown

Comparison of the effects of lipotropic factors It seems advantageous to bring together available information regarding the types of fatty livers which are affected by the three principal lipotropic factors For this purpose it is assumed that with choline may be grouped substances like methionine which enable choline to be synthesized *in vivo*, and also compounds which are related to choline, like triethyl choline The information has been summarized in table 3 Separate literature references are not provided because the activity given in the table is the reviewers' opinion based on an examination of a large number of papers already listed In some cases the activity has been verified in two or more laboratories, such instances are noted with an asterisk In those cases in

TABLE 3

*Comparison of the effects of lipotropic factors*

| REGIMEN USED FOR PRODUCTION OF FATTY LIVERS | CHOLINE | LIPOCAIC | INOSITOL |
|---|---|---|---|
| Depancreatized dogs | ++* | ++* | –? |
| *Rats* | | | |
| High fat diet, thiamin | ++* | 0 | – |
| High fat diet, all B vitamins | ++* | – | – |
| High fat diet, cholesterol | +* | 0 | + |
| Fat-free diet, thiamin | ++* | – | 0 |
| Fat-free diet, thiamin and riboflavin | ++* | – | – |
| Fat-free diet, thiamin, riboflavin, pyridoxine and pantothenic acid | +* | – | +* |
| Fat-free diet, above 4 vitamins and biotin | 0 | ++ | ++ |
| Fat-free diet, B vitamins and cholesterol | + | + | + |

*Note* ++ Strong lipotropic action
+ Moderate lipotropic action
0 No lipotropic action
– Lack of data
* Verified in two or more laboratories

which the activity has been described in one report only there may be some doubt as to the validity of the conclusion

This comparative table has been prepared for several purposes It summarizes existing information in a small space It draws attention to the fact, which has often been ignored, that all fatty livers are not alike in their response to lipotropic agents In doing this, questions may be raised as to the differences which probably exist in the composition of the fats present in the livers It will be noted that in the small number of instances in which comparison has been made there is some correspondence between the effects of lipocaic and of inositol The similarity is possibly not true in depancreatized dogs and there seems to be a distinct difference with regard to fatty livers produced by a high-fat diet containing cholesterol The latter instance is based on one report from one laboratory and there is no confirmation

Why should an alteration in the B vitamin supplements lessen the effect of choline and make it possible for inositol to exert a lipotropic action? Possibly this is due to a change in the fatty acid composition of the liver One might speculate that choline will combine with certain fatty acids to form a phospholipid, while inositol selects quite different acids Some information could be secured by analysis of the two types of phospholipids There is practically no information regarding the mechanism by which lipocaic causes fat to be removed from the liver It should be pointed out that lipocaic and inositol, unlike choline, have a definite effect upon cholesterol metabolism Unfortunately, in the case of lipocaic, there is still no information regarding the chemistry of the active constituent

### SUMMARY

Under normal conditions the amount of fat in the liver is fairly constant An accumulation of excess fat is evidence of an alteration in metabolism In general it may be said that the production of a fatty liver is due to either a failure in transport of fat from the liver or to a rapid withdrawal of fat from body stores at a rate so great that the liver is unable to cope with the fat brought to it Hershey, in his initial studies on fatty livers in depancreatized dogs, assumed that a failure in transport was responsible Accepting the thesis that phospholipids were essential for fat transport, Hershey fed lecithin to his animals and secured a marked reduction in liver fat These results gave rise to extensive investigations, in a number of laboratories, on the cause and prevention of fatty livers Best and associates showed that it was unnecessary to feed lecithin since one constituent of the phospholipid, choline, was sufficient to restore conditions to normal The obvious, and most simple, explanation of the lipotropic action of choline, that it promoted the formation of phospholipids, was validated by the work of Welch and of Perlman and Chaikoff A molecular structure similar to that of choline in general configuration appears to be necessary for the formation of phospholipids of the lecithin type, various substitutions may be made within the molecule with retention of lipotropic activity The effect of protein upon fat transport has been satisfactorily explained as being due to the supply of methyl groups from methionine for the synthesis of choline, for which process ethanolamine must also be available The investigations on transmethylation have shown, as well, that choline may furnish methyl groups for several biological processes This is the second function of choline for which there is definite evidence but it is one which is not related to lipotropic activity A third, and obvious, rôle for choline has been suggested, the formation of acetylcholine There have been described a number of effects of choline deficiency which are not clearly related to any of the three functions ascribed to choline It would seem likely that some of these may be due to the failure of fat transport, this is particularly probable in the case of kidney lesions

While it appeared likely for a short time that choline was the only constituent of pancreas which reduced the amount of liver fat in depancreatized dogs, the work of Dragstedt and associates has established the presence of another lipotropic substance in pancreas, a factor to which Dragstedt gave the name lipocaic

The chemistry and mode of action of lipocaic are unknown In contrast to most early reports, investigations in the reviewers' laboratory have shown that the lipotropic effect of lipocaic can be easily demonstrated in rats, provided that a suitable type of fatty liver is used It should be recognized that all fatty livers are not alike and that the action of a lipotropic agent is conditioned by the character of the lipids in the liver The contention that lipocaic is a hormone has not been substantiated Further investigations, particularly of a biochemical nature, upon the mechanism of production of fatty livers in depancreatized animals and upon the function of lipocaic would be advantageous

It has been established that inositol acts as a lipotropic factor in the rat and it seems likely that this activity is due, like that of choline, to the formation of phospholipids An alteration in the B vitamin supplements will so change a fatty liver that it is resistant to the action of choline but susceptible to that of inositol It would be interesting to determine the changes which are produced in the liver lipids, and which probably determine the activity of the lipotropic agents

While the investigations on lipotropic factors have not, as yet, yielded results which can be applied to the treatment of disease in humans, they have opened a new approach to the study of fat metabolism The chief value of the work on the lipotropic action of choline has been to provide additional proof for the part played by phospholipids in fat transport New information has been made available regarding transmethylation, about the effects of B vitamins upon fat metabolism, and concerning other lipotropic factors The investigations have shown the paucity of our knowledge of fat metabolism and have indicated questions for further research

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# THE BENIGN MELITURIAS

JOSEPH C. BOCK

*Department of Biochemistry, Marquette University School of Medicine, Milwaukee, Wis.*

The term melituria usually designates the appearance of any sugar in the urine in amounts which can be detected by the reagents commonly used for the purpose. Small amounts of reducing substances are always found in minute quantities, but their presence can only be detected by the use of special methods. It is questionable whether these substances are hexoses. Neuwirth (46) and others have observed that some of the material is fermentable in part. Peters and Van Slyke (49) came to the conclusion that normal urine does not contain more than 0.002 per cent of glucose and that the rest, reactive to alkaline copper solutions, consists of substances other than glucose, presumably glucuronides, ascorbic acid, phenols and unknown carbohydrates of possible ketone nature.

The quantitative appearance of any substance in the urine depends on its renal threshold. Due to the fact that this paper deals, to a considerable extent, with the glycosurias, this renal function is briefly discussed. The threshold for glucose is commonly given as 150 mgm. to 180 mgm. In a series of blood sugar studies by this author and co-workers (6, 19, 7) it was found that the accepted maximum can be considerably exceeded (220 mgm.) without glycosuria becoming evident. Benedict and Osterberg (4) consider the glucose threshold "wholly an artifact." They admit that the causes leading to hyperglycemia and subsequent glycosuria are usually the same, but question the necessity of a causal relationship of the two phenomena. In an earlier paper the same authors (3) claim that "the normal organism is truly diabetic in that it has no absolute tolerance for carbohydrate." For further information, the reader is referred to the very thorough discussion of this question by Macleod (35) and to the work of Campbell and collaborators (9).

The proposal of Lusk (34) that we must distinguish between glycosurias caused by an overstrained *sugar holding capacity* of the organism on one hand and the glycosuria of diabetes mellitus, due to an impaired *sugar burning* function on the other, will be of help in the classification of the various meliturias.

*Alimentary glycosuria* becomes evident whenever the sugar holding capacity of the body is exceeded. This capacity is influenced by the dietary habits of the individual, i.e., it depends on the question whether the habitual intake of carbohydrates is large or small. It is a well known fact that the ingestion of a given amount of glucose, followed by an equal dose after a certain amount of time, produces first a rise in the blood sugar which is followed within an interval of one hour or so by a fall. This is known as the Staub-Traugott (58, 60) effect, although it was observed earlier by Hamman and Hirschman (22). In attempt-

ing to diagnose an alimentary glycosuria resulting from a supposed overloading, we must ascertain if the excessive intake took place on a fasting or nearly empty stomach It is known that alimentary glycosuria is more apt to occur under this condition as observed by Goldblatt and Ellis (20)

According to Joslin (25), the following precautions should be taken whenever alimentary glycosuria is encountered 1, correct diagnosis, 2, medical supervision, and 3, blood and urine tests at least yearly

*Renal glycosuria*, erroneously referred to as renal diabetes or diabetes innocens, is a condition in which urinary glucose is found throughout the day even in the overnight fasting specimen It is associated with normal carbohydrate utilization as expressed by the R Q The amount of sugar is largely independent of the diet and the threshold is low (56–100 mgm ) The condition is in all probability due to an impairment of the tubule cells, affecting the function of reabsorption of the sugar. Phlorhizin produces renal glycosuria It also inhibits markedly the phosphorylation processes of the body Because of these facts, the conclusion presents itself that the diminished resorptive power of the kidney in renal glycosuria is due to a disturbance of the phosphate exchange The condition is rare Joslin and co-workers (25) report the incidence of 62 cases in 18,000 melliturias, 9 of the above being renal glycosurias of pregnancy Fowler (16) found 7 among 4000 and Marble (39) records 16 renal glycosurics in 9000 patients with urinary sugar

It is extremely doubtful that renal glycosuria may progress to diabetes mellitus Joslin (25) had 45 cases under observation for a period of 10 years or more Not one of these developed diabetes mellitus In one of the cases reported, sugar in the urine was discovered at the age of ten, in quantities rising occasionally to 5 per cent or more The person was 58 years old in 1940, is on a slightly restricted diet, in good health, the father of five normal children

The *renal glycosuria* of pregnancy was observed by Frank and Nothmann (17) and by Nürnberger (48) Pillman-Williams and Wills (50), as well as Rowe, Gallivan and Matthews (52), report the appearance of urinary glucose of benign aspect during the antepartum period in 35 to 60 per cent of the cases studied The blood sugar levels were nearly normal, with a tendency to fall as pregnancy progressed Only its impermanence distinguishes this type from ordinary chronic renal glycosuria The condition has been used as the basis of a pregnancy test by Welz and Van Nest (62)

When renal glycosuria is suspected, carbohydrate tolerance tests and identification of sugar are of prime importance Dietary rules should include a restricted intake of actual sugars, with directions for a liberal caloric sustenance The patient should remain under his physician's care until the harmless character of the condition is firmly established The normoglycemic glycosuria of pregnancy should receive special attention because cases have been reported where diabetes mellitus developed in the second pregnancy

*Emotional (psychic) glycosuria* As early as 1878 it was observed that excitement or pain caused the appearance of sugar in the urine Böhm and Hoffmann (8) found that when cats not under anesthesia were tied to the operating table,

and when tracheotomy was performed, glycosuria of several hours' duration was observed. They called this phenomenon "Fesselungs Diabetes." Cannon and his co-workers (10) found urinary sugar in cats following fright or rage. However, under the same experimental condition, no glycosuria was noticed after adrenalectomy. This observation conjoined with the fact that normal animals showed an increase in epinephrine secretion under excitement pointed to the influence of the adrenals on the blood sugar level and carbohydrate metabolism. Cannon first used the term "emotional glycosuria."

Folin and associates (15), as well as Malmirwirta and Mikkonen (36) found glycosuria in students after course or subject examinations, the percentage of glycosuria in the group increasing with the severity and importance of the tests. Psychic or emotional glycosuria was observed in soldiers under fire, as cited by Mieth (43) and by Marañon (38) in aviators. Cannon (11) found urinary glucose not only in football players actively participating in the game, but also among the substitutes on the bench, as well as in spectators. Mann (37) observed transient glycosuria when patients were informed that they would have to undergo surgery. Kooy (27) explains the glycosuria of melancholia and manic excitement by the fact that the emotional state is responsible for the high blood sugar, the melancholic depression differing from normal grief by being mixed with anxiety, and manic excitement varying from normal joy because of its association with irritability.

A doctor's thesis by Mieth (43) is of decided interest in the present problem. It deals in detail with the psychic influences on carbohydrate metabolism not only from the position of sex, family or race, but also from the standpoint of occupational factors.

Emotional glycosuria of transitory nature can certainly be called harmless. If, however, the underlying cause is a state of prolonged worry and anxiety then the problem must be considered from the possible "diabetogenic" angle. Von Noorden (47) points to the fact that "high strung" people are more apt to become diabetics than the phlegmatic type. Lichtwitz (33) classifies certain vocations as "diabetogenic professions", i.e., callings where the productive attempt is coupled with psychic excitement and heavy responsibilities. The wealth of observations relative to the psychic or emotional influence on the metabolism of sugars should serve to remind us that the mental state of the diabetic must receive serious attention relative to the proper management of the disease.

*Lactosuria.* An increase in the reducing power of the urine of a pregnant woman was first observed by Lehman in 1850 (31). Hofmeister (23) obtained from the urine of a parturient woman a crystalline substance which had all the properties of lactose. Watkins (61) and later observers find that lactosuria occurs in varying intensity toward the end of pregnancy. A sudden marked increase of lactose is noted during the last two or three days before delivery, the maximum level being reached at the time of parturition. After delivery there is an immediate drop and this low level is maintained from two to six days. This is then very frequently followed by an abrupt and often tremendous increase in the sugar elimination, with subsequent marked fluctuations, lasting for about one month

Thereafter, the urine lactose assumes a lower and more constant level The subsequent downward trend is usually augmented by the weaning process It might be of interest to mention that the tolerance to lactose is increased during menstruation In the intramenstrual period it is the same as that of normal men, the tolerance dose being about 10 grams

*Galactosuria* has been observed in sucklings and in patients following a galactose liver function test In infants as well as in adults, the condition has been known to occur but always under pathological conditions usually involving the liver or the spleen Such observations are found in the publications of Göppert (21), Bansi (2) and Mason and Turner (42)

*Essential fructosuria* (*levulosuria*) is a rare abnormality, a benign congenital error of carbohydrate metabolism In normal individuals 80 per cent of the ingested fructose is converted to glycogen, the remainder going to lactic acid In a case of fructosuria, 10 to 20 per cent of the ingested levulose is excreted instead of being metabolized According to Sachs and collaborators (53) and to Silver and Reiner (56) the disturbed fructose metabolism is possibly due to the lack of a specific enzymatic action of unknown location, probably in the liver, intestine or blood Jacobson (24) ascribes the phenomenon to an increased permeability of the kidney to this particular sugar Fructose is less rapidly absorbed from the intestine and is utilized at a rate of only one-tenth of that of glucose Soisalo (57) determined the fructose tolerance of three normals giving 1 gram of the sugar per kilogram of body weight He found the highest level at 5 to 6 mgm per cent and the renal threshold just below this figure Joslin (25) gives a threshold value of 11 mgm per cent (1 case)

Fructosuria was first observed by Zimmer (63) and at the present date there are approximately 60 cases reported in the literature It is generally assumed that there is only one fructosuric in more than 100,000 individuals Joslin (25) reports three cases in a study of 18,000 melliturias A thorough review of the most typical cases can be found in the work of Sachs and co-workers (53)

The condition persists throughout life, the fructose being found in the urine in a fixed relationship to the intake If a suspected fructosuric is given glucose there will be no, or very little, increase in the reducing power of the urine whereas if the fructose intake is augmented, the rise in urinary sugar can be noticed within a short time by semi-quantitative or quantitative tests (Benedict's) This simple procedure may be of help where only the simplest laboratory facilities are available

The probable existence of a hereditary factor has been mentioned by some observers Lasker (30), after a careful study of this problem, comes to the conclusion that "three classes of evidence indicate that fructosuria is inherited as a Mendelian recessive fructosuria is common among brothers and sisters, it is absent in the parents and children with fructosuria, and parents are frequently consanguineous "

To establish a positive diagnosis we must have levo-rotation on polariscopic examination, absence of reduction of the usual sugar reagents and no optical activity after fermentation, a normal dextrose tolerance curve and identification

of the fructose by special tests. The Selivanoff test (55) is the most widely known. While this procedure is reliable in the hands of the experienced technician, it must be remembered that the typical red color can also be obtained with glucose if the heating is too prolonged. A test that can be performed with the simplest equipment is described by Lasker and Enklewitz (28). The procedure is as follows: 1 ml. of urine is added to 5 ml. of qualitative Benedict's reagent in a test tube. The mixture is heated in a water bath for ten minutes at 55°C. A yellow precipitate forms if fructose is present. Glucose does not reduce under these conditions. A white precipitate may be due to phosphates. Pentoses (xylulose) will also react, but can be ruled out, if necessary, by special tests. Furthermore, the possibility that fructosuria and pentosuria could be present in the same individual is exceedingly remote. Quantitative tests have shown as high as three and one-half per cent of fructose, although the amounts commonly found are much less.

Fructosuria has supposedly been observed in rare cases of severe diabetes mellitus, one such case is described by Marble and Smith (40).

*Pentosuria or xylulosuria* is connected with the appearance of a five carbon sugar in the urine. The pentoses excreted are not the same as those found in various chemical combinations in the body tissues, such as ribose and desoxyribose, but in most cases are xylulose, formerly called l-xyloketose. The condition is of no clinical significance since the metabolism of carbohydrates is not impaired.

The mechanism that produces the most commonly occurring urinary pentose, i.e., xylulose, is not definitely known. Joslin (25) states that the output is increased following a diet high in plums, cherries, prunes and grapes. Enklewitz and Lasker (14) fed a pentosuric 5 grams of xylulose isolated from the subject's urine. The output of the sugar was increased by only 0.5 gram. A normal control showed no reducing sugar after the same dose. The authors conclude that both the pentosuric and the normal can metabolize xylulose when administered orally.

It may be that the mother substance will be found through a study of the glucuronic acid metabolism. Certain glucuronogenic drugs such as amidopyrin, codein, menthol and phenobarbital increase the sugar output whenever pentosuria prevails. These same substances also further the formation of glucuronic acid. If glucuronic acid itself is fed to a pentosuric, we find an increase in the output of the sugar. There is a close chemical relationship between glucuronic acid and xylulose, the latter is derived from the former by the splitting of one molecule of carbon dioxide.

The first case of pentosuria was described by Salkowski and Jastrowitz (54) who found the sugar in the urine of a morphine addict. Neuberg (45) isolated and identified a racemic arabinose in a urine. Some of the older investigators have reported arabinose and other pentoses, but with the advent of more modern methods, we must agree with Levene and LaForge (32) that the sugar occurring in pentosuria is most always xylulose and very rarely racemic arabinose.

All reported cases of pentosuria with one possible exception (44) have been

Jews or people of distinct Jewish lineage, with the males predominating. The earliest "onset" is reported by Garrod (18) in a child of eighteen months. Pentosuria is found in all ages and persists throughout life. Margolis (41) finds an incidence of eleven cases in 22,000 persons examined. Blatherwick (5) found about one in 50,000. Joslin (25) discusses nine cases, all of them Jewish, ranging from three years to thirty-two years in age, with seven males to two females. Five of these cases have been under observation for ten years or more, all with persistent melituria, but in good health and with no sign of progress toward diabetes mellitus. Only one of the above cases showed a blood sugar which was occasionally above normal, but practically no other symptoms indicative of true diabetes. There seems to be a preponderance of males among pentosurics. It must be remembered, however, that many of these are discovered among applicants for life insurance, where males outnumber the females. Among children under fifteen years of age, the literature reports seventeen boys and eight girls.

Pentosuria is of distinct familial tendency. Af Klercker (1) pointed to this possibility as early as 1912. Lasker and co-workers (29) after an exhaustive study of this problem, come to the conclusion that the conditions which permit or cause xylulosuria are inherited as a simple recessive anomaly. It is doubtful that there may be other contributory factors. Protas (51) suggests that nervous trauma or infection may be contributory, but there is a possibility that the factor of drug action was overlooked in the study of the problem.

There are indications that pentosuria may occur jointly with mild diabetes mellitus. Earlier workers report this observation, but at the same time admit difficulties in the positive differential identification of the sugars present. Moss (44) studied the case of a non-Jewish woman. Over a period of eleven days her glucose excretion varied from 0.00 gram to 3.04 grams, and the pentose from 0.7 gram to 2.4 grams.

The presence of both sugars complicates the management of the diabetes, because of hypoglycemic manifestations occurring with reducing sugar in the urine. Insulin has no influence on the pentose metabolism. Edelman and Reiner (13) describe two cases (Jewish males), one of them a mild diabetic, as indicated by glucose tolerance tests.

To arrive at a diagnosis of pentosuria we must have a positive identification of the sugar, an absence of clinical manifestations of diabetes mellitus, a normal glucose tolerance curve and, if possible, a history of pentosuria in the family. To differentiate between pentoses and other reducing sugar, the Bial test is commonly used. A green color is produced by pentoses. The color can be shaken out with amyl alcohol and a typical absorption band near the D line is observed on spectroscopic examination. A confirmatory test with aniline acetate paper is recommended. Glucuronates may simulate a positive pentose test with Bial's reagent. They can be removed by treating the urine with Merck's blood charcoal. As mentioned before, pentoses will react like fructose if the procedure of Lasker and Enklewitz (28) is followed. It must also be remembered that a urine containing xylulose will retain its reducing power almost indefinitely, whereas a glucose urine, unless very acid, will show a greatly diminished reduction or no

reaction to Benedict solution at all, if allowed to stand at room temperature or in an incubator overnight The naphto-resorcinol reaction of Tollens (59) distinguishes between pentoses and glucuronides

Fermentation methods may prove of distinct help in the identification of the sugars encountered in the various meliturias It has been found that so-called baker's yeast, sometimes used for the purpose, is usually not pure enough Other mycologic agents, such as the monilias and certain sugar fermenting bacteria, can be obtained in pure culture and have been found very useful For details, the reader is referred to the publications of Castellani and Taylor (12) and of Klein (26)

There are or may be a number of additional substances in the urine which give or simulate a positive Benedict reaction Maltose has been reported in the urine of beer drinkers No positive indentification of the sugar has been made The phenomenon could be due to a deficiency or absence of the widely-distributed enzyme maltase Alkaptone bodies will give a reduction with the alkaline copper reagents The supernatant fluid, however, is brown or greenish brown instead of blue Bismuth reagents are not reduced and there is no fermentation by yeast Some derivatives of glucuronic acid will reduce Benedict's reagent. The glucuronides or benzoic or salicylic acid are hydrolyzed by alkali, the phenolic derivatives are not

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# EXTRINSIC FACTORS THAT INFLUENCE CARCINOGENESIS

HAROLD P RUSCH

*McArdle Memorial Laboratory, Medical School University of Wisconsin Madison*

One of the most fundamental discoveries in the entire field of cancer research is the observation that certain chemical or physical agents are capable of inducing tumors when brought in contact with living tissue This discovery was followed by extensive experimentation and by the accumulation of voluminous data on the production of neoplasms with the various carcinogenic agents Detailed studies have been made of the relation of chemical structure or of specific wavelengths of radiant energy to carcinogenicity, of the elimination of chemical carcinogens from the body of the variations observed among species, strains and tissues, of age, sex, heredity, hormonal and extrachromasomal influences, and of the extraneous physiological effects of the carcinogen Most of this information has been summarized previously (34 52, 54, 55, 75, 81, 87)

The purpose of the present review is to assemble the information available on various extrinsic factors that alter the action of the carcinogens No attempt has been made to include experiments dealing with established neoplasms In general, in studies of this type, animals are treated with a carcinogen and in addition some other factor is administered by any of several methods The studies appear to be motivated not only by academic curiosity but also by the hope that sufficiently extensive information on factors that alter carcinogenesis might lead to an understanding of the carcinogenic process itself or, in other words, to the cause of cancer

This objective is still far away, and from the larger point of view the available data, though extensive are indeed quite fragmentary One fundamental question of interpretation can be raised against each of the experiments discussed To what extent is the observed result concerned with the carcinogenic reaction itself and to what extent is it merely an expression of a change in the metabolism of a specific carcinogen? To illustrate, the injection of benzpyrene dissolved in mouse fat results in fewer tumors than when it is injected in a vegetable oil (65) This might indicate the presence of an anticarcinogen in mouse fat, but it is equally possible that a more rapid elimination of solvent with a corresponding quickened removal of the carcinogen occurs when mouse fat is employed Accordingly, the results cannot be properly evaluated until the means are available whereby the necessary distinction can be made

Extrinsic factors may either accelerate or retard the formation of neoplasms, and they may be effective just prior to, during or following the administration of the carcinogenic agent. Thus phrases such as "the production of those biological changes which represent the latent period of carcinogenesis ' "the preparation of the soil,' "the precipitation of a tumor at a site previously rendered neoplastic" have come into use For the purpose of the present discussion the terms proposed by Berenblum (20) will be followed

*Anticarcinogenic action* The inhibition of the process of carcinogenesis

*Cocarcinogenic action* The augmentation of carcinogenesis by a noncarcinogenic agent

This occurs when the appropriate agent is applied concurrently with a carcinogen which is acting under suboptimal conditions

*Precarcinogenic action* The production of a preneoplastic condition Such an effect would be demonstrable by a shortening of the latent period of carcinogenesis in subsequent treatment with a carcinogen, or by preparing the ground for the subsequent action of an epicarcinogenic agent

*Epicarcinogenic action* The production of tumors in a tissue previously rendered preneoplastic

*Metacarcinogenic action* The conversion of a benign into a malignant tumor

As might be expected, the dose of carcinogen is extremely important in determining the rate of cancer production, and extensive information on this subject is available not only for the hydrocarbons (41, 42, 45, 55, 70, 75, 87, 113, 183), and for the azo dyes (5, 96, 132, 185), but also for ultraviolet irradiation (11, 35, 36, 37, 181) Unfortunately for a study of extraneous factors on carcinogenesis, doses frequently have been selected for the production of the maximum number of tumors in the shortest time, a technique obviously not suited for testing the influence of factors that might cause some alteration of carcinogenesis Several investigators have shown that the effect of certain agents might be obscured when overwhelming doses of carcinogen were employed (13, 45, 65, 183, 197) A satisfactory procedure is to administer the carcinogen at a level that will induce tumors in about 50 per cent of the animals If the agent has been previously shown to have an augmenting action, it is desirable to test its effect on carcinogenic doses which by themselves induce less than 50 per cent tumors, whereas if a known inhibitor is used, the dose of carcinogen can be increased somewhat

THE INFLUENCE OF AGENTS ADMINISTERED DIRECTLY TO THE REGION OF CARCINOGENESIS *A Chemical Agents Solvents* One factor that can influence the carcinogenicity of a hydrocarbon is the medium in which it is dissolved There appears to be good agreement that the development of neoplasms is retarded when crystalline hydrocarbons are injected subcutaneously by methods that leave a deposition of crystals within the tissues Various investigators have shown that the injection of hydrocarbons as powders, pellets, or as crystals moistened with a lubricant produce tumors less rapidly and in fewer animals than when given in oil solution (6, 7, 162, 201) Furthermore, the incidence of tumors was lower and the latent period longer when hydrocarbon-cholesterol pellets were introduced into the subcutaneous tissues of mice (186, 187), but it is difficult to make direct comparisons with other methods because of the different amounts of hydrocarbon that come in contact with the tissues when cholesterol pellets are employed Peacock and Beck (162) believe that the induction of sarcomas following the injection of hydrocarbons in various solvents is chiefly dependent upon the rate of absorption of the carcinogen

Hydrocarbons have been applied to the surface of the skin in many solvents, but no marked differences have been observed among the common organic solvents (53) and benzene seems to have the greatest popularity, although dioxan (109), acetone (29) ether plus mineral oil (57), and benzyl alcohol (182) have the advantage of being less toxic than benzene Twort and Twort (205) ob-

served a stimulation in the rate of carcinogenesis with dibenzanthracene, methyl cholanthrene or benzpyrene when chloroform was used as the solvent. Crabtree (56) found that the average induction time for papillomas was significantly lower when ether plus 2 per cent of liquid paraffin replaced benzene as a solvent for benzpyrene or when acetone plus 2 per cent paraffin was used instead of benzene as the solvent for dibenzanthracene.

*Fats and oils.* Various fats and oils are among the most common substances employed as solvents for the carcinogenic hydrocarbons and a pronounced influence on tumor genesis has been observed with these substances. The incidence of tumors is high following the subcutaneous injection of carcinogens dissolved in vegetable oils (corn oil (13), cottonseed oil (179), sesame oil (190), arachis oil (10), olive oil (162)), synthetic glycerides (188), lard (6), or paraffin (69), whereas fewer neoplasms are produced when fatty extracts of animal tissues are used as the solvents (49, 65, 145, 151, 162, 212). Lard, an animal product, appears to be the single exception to this general rule.

Peacock and Beck (162) reported that the injection of 0.5 to 1 mgm. of benzpyrene dissolved in mouse fat produced only a few tumors whereas carcinogenic activity was pronounced when the solvent was olive oil, or a mixture of ¾ olive oil and ¼ paraffin. Morton and Mider (145) obtained only 1 tumor in 44 mice that had received subcutaneous injections of 0.25 mgm. of benzpyrene dissolved in a petroleum extract of mouse carcasses, while the same amount of hydrocarbon dissolved in sesame oil produced 36 tumors in 46 mice. Dickens and Weil-Malherbe (65) observed that the subcutaneous injection into mice of solutions of 0.3 mgm. of benzypyrene in sesame oil or in arachis oil gave rise to tumors in a large proportion of the mice, while the same dose of benzpyrene in either the liquid or the solid fraction of mouse fat produced only a few tumors. For the suppression of carcinogenic activity, it did not appear necessary that the lipoid material be of homologous origin since a mixture of fats and lipids obtained from ox brain was equally effective (65).

On the contrary, Oberling and his collaborators (156, 157) and Shimkin and Andervont (188) did not observe any retarding effect of rat or mouse fat. The latter workers, however, used comparatively large amounts of carcinogen: 25 to 75 mgm. of benzpyrene (156, 157) or 0.5 to 1.0 mgm. of methylcholanthrene (188). More recent work by Bryan and Shimkin (42) and by Sall and Shear (183) indicates that about 0.02 mgm. of methylcholanthrene is sufficient for the development of tumors in 50 per cent of their mice.

Peacock and Beck (162) attribute the anticarcinogenic action of homologous fat to the more rapid elimination of the solvent from the injection site, with a corresponding rapid removal of the carcinogen. Certain observations of Dickens and Weil-Malherbe (65) while not necessarily contradicting this explanation, nevertheless fail to support it: *a*, the persistence of subcutaneous lumps near the injection site of the liquid mouse fats apparently indicating a very slow dispersion of the injected solution, *b*, the fact that the solid fraction from mouse fat, which might be expected to be even less readily dispersed and which also gave rise to many persistent lumps was similarly effective in preventing tumor

formation" These investigators imply that certain animal fats contain substances that are anticarcinogenic

Another explanation is that the differences observed in tumor incidence when the hydrocarbons are dissolved in various fats are due to varying amounts of ingredients that augment or retard the carcinogenic process itself In a very extensive and careful study of the effect of solvents on carcinogenesis due to hydrocarbons, Leiter and Shear (113) found differences in tumor formation between two different fractions of good quality pure leaf lard The lard was filtered at 38°C for 24 to 36 hours through coarse paper The fraction that was liquid at 38° and which passed through the paper was designated "lard filtrate," and the material remaining on the filter paper was designated "lard residue" When the lard filtrate was used as a solvent for benzyprene the incidence of tumors varied widely although tumors usually developed in a high proportion of the animals The variations were attributed to different proportions of retarding and promoting substances in the various specimens of lard filtrate employed Lard residue exercised a striking retardation of tumor genesis by benzpyrene Fractionation of lard, roughly according to degree of saturation of the fatty acids, indicated that the more saturated samples caused the greatest inhibition of carcinogenesis Furthermore, tristearin and tripalmitin also inhibited tumor formation (113)

Still another explanation for differences in tumor incidence with various fats was suggested by the observation that benzpyrene dissolved in mineral oil or in vegetable oil survived oxidation *in vitro* for many weeks, but that in the presence of linoleic acid, the hydrocarbon had almost entirely disappeared within three weeks (150) The addition of $\alpha$-tocopherol to the linoleic acid greatly increased the time required for the disappearance of the hydrocarbon Vegetable oils contain considerable amounts of antioxidants and their stability to autoxidation is well known (158), whereas animal fats contain only small amounts of inhibitols and are quite susceptible to rancidity (158) Thus a loss of benzpyrene might occur when it is dissolved in an ether extract of animal tissues The injection of such a mixture would in effect, then, only result in the administration of less carcinogen On the other hand, when a stable oil was used as a solvent, little destruction of the hydrocarbon occurred The successful production of neoplasms with lard as a vehicle for the hydrocarbons suggests the possibility that lard is more stable to oxidation than are the ether extracts of animal tissue It would seem wise to test this point

Apparently the effect of fats on the genesis of tumors is a complex one and no single theory covers all the known facts Furthermore, generalization is difficult because the influence of certain fats may vary according to the technique of administration Thus, Watson (212) demonstrated that pinene tar mixed with an ether extract of rat tissues augmented the formation of skin tumors when applied to the surface but prevented tumor genesis when injected subcutaneously Watson and Mellanby (213) painted the skin of mice with a petroleum ether extract of mouse fat before tarring and observed an enhanced formation of tumors Morton and Mider (146) also noted that the "fat effect" varied with

the mode of administration When a petroleum ether extract of mouse carcasses was used as a solvent for benzpyrene which was injected subcutaneously or applied directly to the skin, tumor formation was retarded, but when this same mouse fat extract was painted on the skin 20 to 30 minutes prior to the application of benzpyrene, formation of skin tumors was augmented Baumann, Rusch and co-workers (15, 91) observed that tumors were formed faster when 0.3 per cent benzpyrene was applied to the skin in a solvent containing cholesterol in cottonseed oil than when benzene was used as the solvent In a continuation of this problem, Lavik and Baumann (110) tested the effect of other fatty substances on the skin of mice that were also being painted with a solution of 0.2 per cent methylcholanthrene The carcinogen was applied twice weekly for the first 2 months of the experiment, and either oleic acid, potassium oleate solution, cottonseed oil, or a 5 per cent solution of cholesterol in cottonseed oil was applied at the site of carcinogenesis on days when the hydrocarbon was not painted. A definite increase in tumor incidence resulted from the local applications Twort and Twort (205) reported that oleic acid augmented the development of skin tumors and suggested that cells rendered abnormal by a few applications of benzpyrene quickly passed into the irreversible cancerous phase when stimulated by this fatty acid Contrary to these findings, oleic acid has been shown to inhibit the activity of the chicken tumor virus (88) Tumor growth has also been inhibited by the injection of fatty acids (19, 152) In this connection, it would be interesting to test the effect of the more unsaturated linoleic acid on tumor formation

Mineral oil, in contrast to most oils of biological origin, did not augment the genesis of skin tumors, and when this substance was used as a solvent for the surface application of methylcholanthrene it actually retarded carcinogenesis somewhat (176, 205) Recently Simpson and Cramer (189) noted that the carcinogenic activity of methylcholanthrene was almost completely suppressed when dissolved in anhydrous lanolin and applied to the skin, thus confirming an earlier recommendation of lanolin as a protective agent against tar dermatitis (204) Lanolin is a mixture of variable composition and is known to contain phospholipoids and sterols as well as neutral fats Since cholesterol alone has little influence on carcinogenesis either of the skin (176) or of subcutaneous tissues (186), studies of the effect of phospholipids on tumor genesis appear to be indicated

Certain oils applied to the ears of mice exposed to ultraviolet radiation caused a very considerable acceleration of tumor development (170) mineral oil, and cholesterol in cottonseed oil were particularly effective, cottonseed oil, olive oil, and wheat germ oil gave moderate acceleration, while linseed oil retarded tumor formation The retarding effect of this latter oil was ascribed to the formation of a film of oxidized oil which decreased the penetration of the carcinogenic light Because peroxide formation has been observed when cholesterol and certain oils are irradiated in air, two peroxides, benzoyl peroxide and hydrogen peroxide, were applied to the ears of mice receiving irradiation, but they were without effect on carcinogenesis Glycerol was also ineffective Furthermore,

none of several irradiated oils had any carcinogenic properties The accelerating effect of the oils on carcinogenesis due to light was ascribed to the presence of a thin film that formed a smooth surface on the ears and thus decreased the reflection of light from the skin Presumably under these conditions, more radiant energy penetrated to the deeper layers of the tissues However, it is also possible that the activity of the cells themselves was altered by the presence of oil (176)

Virtually all known carcinogenic chemicals are soluble in fat, and the carcinogenic hydrocarbons are capable of inhibiting the autoxidation of fats *in vitro* (63, 64, 178) They might also interfere with fat oxidation within the cell Furthermore, the fats, as a group, appear to be more effective in influencing the activity of a wider range of carcinogens than any other type of agent It would appear, therefore, that no theory which attempts to explain the mechanism of carcinogenesis can cover all the facts unless some phase of fat metabolism is included within it

*Irritants* Another group of substances that affect the formation of tumors might be designated as "irritants" although not all irritating substances affect carcinogenesis Irritation, itself is not an easily definable entity "It is, in fact, a vague generalization representing many different kinds of injury or stimulation of the tissues Attempts have been made to classify irritants according to their action on the epithelium, hair follicles, blood vessels, nerve endings, etc , and according to whether the effects on the epithelium are growth stimulating, astringent, desiccating, or emollient Owing to the complexity of action in most cases, such attempts at classification have generally proved confusing and are often definitely misleading" (27)

Earlier writers have frequently considered irritation as a carcinogenic agent but recent evidence suggests that it is merely a contributing factor in carcinogenesis Croton oil, a powerful irritant, happens to be one of the most potent cocarcinogens known Berenblum (28) applied an acetone solution of 0 05 per cent benzpyrene to mice at weekly intervals and observed tumors in only 3 per cent of the animals This incidence was increased to 37 per cent when 0 5 per cent croton oil was added to the solution of benzpyrene Croton resin, (50) a constituent of croton oil, possessed cocarcinogenic action to an even greater degree, the incidence of tumors was increased to 80 per cent when the resin was applied with the benzpyrene Both the croton oil and resin were demonstrated to be themselves devoid of even slight carcinogenic activity The croton resin also precipitated tumor formation in areas previously treated with benzpyrene (29) A group of mice received 8 weekly applications of a 1 per cent solution of benzpyrene in acetone and the animals were then divided into three groups In the first group, the skin was painted at weekly intervals with a 0 025 per cent solution of croton resin in acetone, in the second group, the skin was painted with 30 per cent of turpentine in acetone, while in the third, acetone alone was applied as a control The painting was continued for 22 weeks, after which the animals were left untreated for a further 6 weeks when they were killed for histological examination In the acetone control group 18 per cent had developed tumors

at the site of application, in the turpentine group 44 per cent of the mice had tumors, while those receiving the croton resin showed a tumor incidence of 86 per cent Furthermore, fewer tumors regressed and more became malignant in the group receiving the croton resin than in the control group, while the turpentine series was intermediate

Creosote oil is another substance that influences tumor formation, although, unlike croton oil, which acts only as a positive promoter of tumors, creosote oil has been separated into fractions three of which augment while four others retard neoplastic formation due to benzpyrene (45, 183) The retarding agents also caused damage to the skin and this might have been responsible for the retardation, however, a phenolic fraction which was anticarcinogenic produced no obvious injury to the epithelium These fractions were tested by applying them to the mice simultaneously with the hydrocarbon, it would be of interest to determine their effects when administered after a preliminary treatment with a carcinogen.

As an example of an irritant that does not affect carcinogenesis, xylene has been applied to mouse skin in concentrations producing an irritation equivalent to that due to croton oil, without, however, altering the number of tumors that developed as a result of benzpyrene treatment (28) Although turpentine possesses weak epi and metacarcinogenic activity (29), it, like xylene, was virtually devoid of cocarcinogenic activity for skin tumors caused by benzpyrene applied during the first phase of tumor formation (28) Furthermore, when benzpyrene was dissolved in a 30 per cent solution of turpentine in olive oil and injected subcutaneously into mice, abscesses were induced around the injection mixture, but this acute inflammatory reaction did not accelerate the genesis of tumors On the contrary it appeared to diminish the incidence of neoplasms (16)

Still another group of chemicals that has a pronounced influence on carcinogenesis includes mustard gas and related compounds Berenblum (24, 26) observed that the addition of 0 05 to 0 1 per cent of mustard gas ($\beta,\beta'$ dicholoro diethyl sulphide) to a carcinogenic tar inhibited tumor production Only 18 out of 240 mice developed tumors when a mixture of tar and mustard gas was applied to the skin, in contrast to an incidence of 131 out of 235 when tar alone was used under identical conditions Of the compounds related to mustard gas, ethylene bis-chloroethyl sulphide and dibromodiethyl sulphide, gave marked inhibition, dichlorodiethyl sulphone and dibromodiethyl sulphone gave moderate inhibition, while diiododiethyl sulphone, dichlorodiethyl sulphoxide, thiodiglycol and dichlorodiethyl ether were without effect (27) Among the irritants not belonging to the mustard gas group only cantharidin gave marked inhibition while cyclohexane elicited a slight retarding effect Iodoacetic acid, trichloracetic acid, croton oil, acetic acid, "lysol", and turpentine were without influence

Mustard gas is an oily liquid readily soluble in fats and fat solvents but only sparingly soluble in water In the presence of water it is decomposed fairly rapidly into dihydroxydiethyl sulphide (thiodiglycol) and hydrochloric acid It has been suggested that the irritant action of mustard gas is dependent on its high lipoid water distribution coefficient and that the damage is due to the liberation of free HCl inside the cell (118) Furthermore, mustard gas has been shown

to react with amino acids (48) and this suggests the possibility of an interaction with the protein constituents of the cell. Berenblum, Kendal and Orr (30) attempted to determine the metabolic changes induced by mustard gas, etc., but no successful correlation regarding the rate of hydrolysis and the inhibition of metabolic processes could be made. Mustard gas lowered the glycolytic rate considerably while ethylene-bis-chloroethyl-sulfide had little effect on this process.

Crabtree (57, 58) observed that certain concentrations of monochloracetone altered the rate of tumor formation due to benzpyrene, and this "chlor-compound" also inhibited glycolysis. Ninety mice were treated twice weekly with 0.3 per cent of benzpyrene in a solvent of 98 per cent of ether plus 2 per cent mineral oil. To one group of 30 mice 0.3 per cent monochloracetone was applied three times a week, 30 others received 3 per cent monochloracetone in the same way, while the remaining 30 were kept as controls. The low concentrations of monochloracetone caused a marked inhibition of tumor development but the higher levels were either without effect or they stimulated tumor development slightly. Monochloracetone is also capable, in a wide range of concentrations, of stimulating tumor induction in mice that have received a preliminary, but subeffective, treatment with 0.3 per cent benzpyrene applied for 8 weeks. Stearyl chloride, palmityl chloride, myristyl chloride, valeryl chloride, acetyl chloride and benzene sulfochloride when added to 0.1 per cent or 0.3 per cent benzpyrene retarded papilloma formation in mice (58). These latter "chlor-compounds" differ from monochloracetone in that they are hydrolyzable under physiological conditions. Since the one common feature of all these compounds is the active chlorine atom, Crabtree (58) suggested that the inhibition of glycolysis *in vitro* by mustard gas is also caused by the liberation of HCl by this compound.

Reimann and his associates (166) have demonstrated that the application of 0.5 per cent p-thiocresol to the skin of mice will protect it against carcinogenesis due to dibenzanthracene. The application of sulfhydryl compounds apparently speeds the rate of cell multiplication in the basal layer of the skin and with this stimulation, the cells proceed to higher degrees of differentiation and organization. Since neoplasms arise from incompletely differentiated cells, it is suggested that tumor formation will necessarily be decreased in tissues in which most of the cells are well on their way to complete differentiation. Potter (163, 164) has suggested that thiocresol protects the SH groups of certain enzymes that might otherwise be inhibited by carcinogens. Enzymes of both aerobic and anaerobic systems within the cells are known to contain SH groups. It has been suggested that agents that induce shifts from the aerobic to the glycolytic metabolism over a sustained period would, to this extent, duplicate one of the properties of carcinogens (163). Conversely an anticarcinogen might be expected to protect the SH enzymes against inactivation. Certain experimental facts were presented in support of this view. Metabolic split products of p-dimethylaminoazobenzene have been shown to inhibit enzymes concerned with glycolysis (93, 95) and with oxidation (163, 165) presumably by the inactivation of the SH groups. Although it is possible that a similar inactivation of SH enzymes could occur with the hydrocarbons, this has not been susceptible to testing thus

far because of the insolubility of the hydrocarbons in an aqueous medium. It is possible that these latter carcinogens make contact with enzymes which contain some lipoid in the molecule. Certainly more work in this direction is indicated.

Other compounds not classified as irritants have also been shown to influence the genesis of tumors. There is some preliminary evidence that xanthine, hypoxanthine, nucleic acid, histidine, nicotinic acid, d,l phenylalanine, tyramine, tyrosine, and guanine decrease tumor incidence when added to solutions of filtered lard containing benzpyrene (133). The administration of small amounts of indole or arsenious acid to white mice for a period of 45 to 60 days prior to tar painting, has been reported to predispose the animals to neoplasms and to accelerate tumor development (51). Although weak carcinogenic properties have been attributed to arsenic (134) there is little unequivocal evidence to support this view (92). Since the question has arisen concerning the advisability of administering arsenic compounds to individuals under treatment for cancer, it appears that the effect of arsenic on experimental tumor genesis should be reinvestigated.

B. *Physical Agents. Injury, inflammation and healing.* Berenblum (25) has shown that the concurrent application of carbon dioxide snow and tar to the same area of skin inhibited the induction of tumors whereas an augmentation of tumor formation occurred if the treatment with carbon dioxide snow followed that with tar. Since carbon dioxide snow is itself a mild carcinogen (23), this result appeared to represent a summation effect.

Very similar observations were made by DesLigneris (62) who employed heat instead of cold to the skin of mice that also received applications of methylcholanthrene. A small cotton plug mounted on a forceps was plunged into boiling water, left in the air for about 2 seconds, and then applied to the skin by simple contact for about a second. This scalding alone produced no tumors, and when applied on alternate days with the methylcholanthrene, little effect was noted; if anything, the scalding diminished the number of tumors. However, when a three month period of hydrocarbon administration preceded the scalding, the latter treatment precipitated tumors in the preneoplastic area. In fact, the epicarcinogenic activity of the scalding was almost as great as that noted when methylcholanthrene had been continued in its place. Choldin (51) reported an acceleration of tumor genesis in mice if test tubes containing hot water were applied a short distance away from the area treated with tar, although no augmentation of carcinogenesis was noted when the thermal irritation was applied to the treated area. Parodi (101) found that the production of tar tumors was delayed if the skin had previously been heated to 70°C. for several months, while heating the epidermis to 50°C. favored the development of cancer. Lauridsen and Eggers (108) observed a slight acceleration in the development of neoplasms in the skin of mice if the area treated with dibenzanthracene also was cauterized at weekly intervals with a hot wire. The application of a caustic pencil or hot needle to the base of a papilloma stimulated malignant changes in mice whereas incisions of the skin made during the application of tar had no effect on tumor development (59). Brunschwig, Tschelter and Bissell (40) did not find an in-

crease in tumor formation when an area of mouse skin painted with benzpyrene was cauterized with a hot metal object

Injury resulting from scarification or incisions has much the same effect as the trauma produced by thermal methods Deelman and VanErp (61) and Deelman (60) stated that an incision made in the skin of a mouse in the neighborhood of a tar cancer led to the development of a new carcinoma, which appeared in the healing wound These workers concluded that the skin close to a carcinoma is in a precancerous condition, so that the application of a trauma, insufficient to produce malignancy when applied to the skin elsewhere, will elicit the genesis of a neoplasm if applied to the skin in the neighborhood of a tar carcinoma Orr (159) reported that fibrosis produced by placing threads in the subcutaneous tissues underlying the skin treated with dibenzanthracene led to a more rapid appearance of epithelial tumors Ludford (117) used sandpaper to scarify the skin of mice both prior to and following treatment with tar, but observed only a slight retardation of subsequent tumor formation The significance of this observation however, is open to question since many minute tumor nests could have been removed mechanically as rapidly as formed, a result which would be less apt to occur from simple incisions In general it appears that neither thermal nor mechanical injury affects the development of skin tumors if such treatment is attempted too early in the carcinogenic process However, trauma applied after the preneoplastic stage usually stimulates the formation of neoplasms

Injury to the deeper tissues, however, appears to have no effect on the genesis of tumors arising from such areas DesLigneris (62) was unable to accelerate tumor genesis by the injection of glass particles along with methylcholanthrene or benzpyrene Similarly, Boyland and Burrows (38) observed no augmentation when fine silica was mixed with a colloidal aqueous suspension of dibenzanthracene Woglom (220) injured glandular epithelium by drawing threads soaked in benzpyrene through various animal organs but this trauma plus hydrocarbon did not induce tumors in the various organs

A rapid vigorous growth of liver tumor cells in healing wounds, which was soon followed by a regression of the neoplasm as the processes of repair neared completion, has been described (101) Three possible explanations for this effect were suggested *a* Tumor growth may be stimulated in an area of rich vascularization but retarded and finally stifled when the blood supply is decreased *b* The injured cells might elaborate one or more factors that stimulate growth Substances of this category have been produced by injured yeast cells (116) and Menkin (129) has shown that an inflammatory exudate injected frequently into the subcutaneous tissue of a rabbit's ear will induce a severe inflammatory reaction Cessation of these injections is followed months later by a sustained and marked proliferative activity This is characterized by hyperplasia and metaplasia of the normal epithelial layer and by foci of keratinization Furthermore, the cartilage of the ear, at the site of injections, manifested marked proliferative activity giving rise to small nodules *c* Injury to the cells may result in a decrease in local tissue resistance that permits the proliferation of cells otherwise held in check (59, 101)

*Radiant energy* X rays, ultraviolet radiation, visible light and infra red rays all appear to exert some influence on carcinogenesis, and ultraviolet and x ray are themselves carcinogenic

Wallace, Wallace and Mills (210) reported that mice kept at 92°F developed tumors due to methylcholanthrene more rapidly than did mice maintained in an environment of 65°F Bain and Rusch (12) demonstrated that mice kept in a box maintained at 35°C for 30 minutes while exposed to ultraviolet radiation developed tumors faster than controls irradiated at room temperature or at 3–5°C Whether this effect was the result of the increased rate of tissue metabolism or to some other influence cannot be answered at the present time

Visible light also has been reported to influence carcinogenesis with hydrocarbons Doniach and Mottram (68) found that strong sunlight plus benz pyrene painting caused a dermatitis and reduced the incidence of tumors This finding was verified by Morton and his collaborators (143, 144) who used artificial lamps for the source of light Seelig and Cooper (184) were unable to demonstrate any influence of daylight on the effect of tumor genesis in mice painted with tar Conflicting results have been reported by Vles, DeCoulon and Ugo (208, 209) and by Maisin and DeJongh (121) who noted an acceleration of tumor formation when mice painted with tar or benzpyrene were subjected to visible light

The carcinogenic potency of x rays can be augmented if abscess formations are also induced in the treated tissues In a study of this type, Lacassagne (105) and Lacassagne and Vinzent (107) produced inflammation in the subcutaneous tissues of rabbits by the injection of *Streptococcus caviae* or by the injection of sterile diatomaceous earth The lesions were submitted to x radiation, the dose in each instance being 600 r Such doses had a beneficial effect on the infective lesions but sarcomas eventually developed at the sites of irradiation in 5 of the 12 survivors that had the infected lesions and in 2 of the rabbits that had received the sterile diatomaceous earth Burrows, Mayneord and Roberts (44) observed similar results They induced a focus of inflammation in the groin of 12 rabbits by injections of kaolin and of finely powdered silica suspended in olive oil These foci were exposed to 600 r of x ray at a single dose Among nine rabbits thus treated and surviving for two years or longer, tumors appeared in the irradiated tissues of six In four of these instances the tumors were sarcomata that produced metastases X ray alone in the same dose was non-carcinogenic

Büngeler (43) has reported that the incidence of skin tumors in mice due to sunlight can be increased by the injection of certain photosensitizing agents such as eosin and hematoporphyrin Others have suggested that oxidizing agents or substances that alter the oxidation reduction potential might have some influence on tumor growth (176) However the following compounds failed to affect the production of tumors due to ultraviolet radiation rose bengal, neutral red, di chlorophenol indophenol, catechol, p-thiocresol, histamine, benzoyl peroxide, hydrogen peroxide, histamine, benzyl alcohol, and benzene (176 182)

C *Combination of Carcinogens* The carcinogenic effects of the various hydro carbons are apparently additive that is, several different carcinogenic hydro-

carbons may be applied to the skin of mice in almost any order without interruption of the carcinogenic process (89, 111, 182) Apparently skin does not differentiate between the chemicals and responds as if one carcinogen were acting continuously

Variable results are obtained, however, by combining carcinogenic chemicals with radiant energy Mottram (147, 148) observed that the application of radium to the backs of mice previously treated with benzpyrene was followed by a greater number of epithelial tumors This was true only if the dose of radiation was small The tumors were found at a distance from the previous site of the radium applicator, in a region that had received from 800–2800 r units Taschner, Gottlieb and Spritzer (198) treated a series of mice with x-ray taking care to screen an area of the skin with lead After one month a solution of methylcholanthrene was applied to that portion of skin which had been shielded from the x-rays Tumor formation was accelerated in the mice that had previously received the larger doses (650 r ) while the lower dose (150 r ) had a slight retarding effect Brunschwig, Tschelter and Bissell (40) were unable to confirm these results, probably because of differences in experimental procedure The latter workers applied a 1 per cent solution of benzpyrene three times a week for only 2 weeks before the radium plaque was applied, whereas Mottram applied benzpyrene for 60 to 75 days before treating with radium Furth and Boon (80) observed that leukemogenic action of small doses of methylcholanthrene is greatly enhanced by pre-irradiation with doses of x-rays

When ultraviolet radiation was used in combination with tar, increased cancer formation has been reported (76, 200) but these observations have not been confirmed with pure hydrocarbons (176, 182, 199) The treatment of mice with ultraviolet radiation preceding, during or after periods of application of methylcholanthrene or of 9,10-dimethyl-1,2-benzanthracene did not increase the carcinogenic effectiveness of the chemicals, nor did the presence of the hydrocarbons augment the development of tumors due to the ultraviolet radiation (182) Actually there was a slight decrease in the number of neoplasms when both agents were used simultaneously This inhibitory effect could have been due to a partial destruction of the hydrocarbon by ultraviolet light and to the absorption of radiant energy by the hydrocarbon Benzpyrene is known to be unstable to light (149) In contrast to the hydrocarbons, tar is such a complex mixture that light might not readily affect its carcinogenic potency Another explanation for the lack of augmentation between the two types of carcinogens is that they involve essentially different mechanisms that are incapable of summation under these conditions

Observations have also been made on combinations of other dissimilar carcinogens Rous and his collaborators (79, 119, 173, 174) noted that benign warts which had been induced in rabbit's ears by the application of tar became large and invasive after the intravenous injection of Shope papilloma virus, while neither agent by itself induced malignant tumors so quickly Similarly, Ahlström and Andrewes (2) observed an intensified response to fibroma virus when tar was injected intramuscularly However, Rusch and his associates (182)

were unable to demonstrate a summation effect by the combination of Shope papilloma virus and ultraviolet or x irradiation, and Beck (17) did not observe an increase in the general susceptibility to cancer by cauterization, x rays or ultraviolet light when mice were given intraperitoneal injections of benzpyrene

THE INFLUENCE OF FACTORS ADMINISTERED AT A DISTANCE FROM THE REGION OF CARCINOGENESIS A *Diet Caloric influence* A fundamental observation that must be considered in all experiments involving the dietary modification of tumor development is the fact that tumors develop more slowly and in a smaller percentage of animals when the total intake of food is restricted in amount Tannenbaum (195), for example, has shown that the incidence of spontaneous lung tumors in ABC strain mice was reduced by about 50 per cent in animals fed a restricted diet, and a striking reduction in the incidence of spontaneous mammary carcinoma in mice maintained on a caloric restricted diet has also been demonstrated (196, 207) In a group of 50 virgin female mice of the DBA strain fed *ad libitum*, 18 developed spontaneous breast tumors by the 86th week of life whereas no tumors formed in a similar group kept on a diet restricted as to calories (196) The results of Visscher and his co-workers (207) were even more marked 67 per cent of a series of 51 female mice of the $C_3H$ strain allowed unlimited food developed spontaneous mammary neoplasms by the 16th month as compared to no tumors in a similar group fed a diet which was limited in calories by 33 per cent McCay and his associates (127, 128) observed a reduction in the number of spontaneous tumors in rats kept on restricted diets Like spontaneous tumors, several types of tumors due to chemical carcinogens are also susceptible to the effect of a reduced caloric intake Thus the incidence of skin epitheliomas induced in mice by applications of dibenzanthracene was approximately 90 per cent in the full fed control group and only 30 per cent in the experimental group receiving fewer calories, in mice that had received subcutaneous injections of benzpyrene, 82 per cent of the controls had sarcomas as compared to 47 per cent in the restricted group (196) There was also a delay in time of appearance of such neoplasms as developed However, a reduced caloric intake had little or no influence on the subsequent growth of sarcomas induced by benzpyrene or of spontaneous breast tumors (196), although Bischoff, Long and Maxwell (32) have reported a retardation in the growth of sarcoma No 180 in mice whose caloric intake was restricted by 50 per cent, less drastic reductions in food intake failed to yield significant results

The opinion has been expressed that the effects of caloric restriction are observed only in poorly nourished, cachectic animals, and it may be true that in some of the earlier experiments caloric restriction was accompanied by a simultaneous reduction in the intake of protein, vitamins and salts since the diets employed were identical in composition but different in amount in the full fed and restricted groups However, in the more recent experiments cited above (196 207) the restricted mice were fed amounts of protein, vitamins, and minerals, approximately equal to those ingested by mice eating the control diet *ad libitum*, while the carbohydrates and fats were reduced not only in total amount but also in percentage of the diet Thus, the inhibition of tumor formation that

resulted could be attributed to caloric restriction *per se* Some of these experiments were continued for over a year without any obvious nutritional deficiencies appearing in the animals Few other experiments on nutrition have been continued for an equal span of time Although the average weight of the calorie-restricted mice was 20 to 23 grams while the controls weighed 28 to 32 grams, the restricted mice appeared sleek and healthy and, in general, outlived the controls (196) McCay and his associates (128) also noted an increase in the life span of rats kept on restricted diets, but in his experiments, the growth of the rats was deliberately retarded to a gain of only 5 grams in 50 days

The interesting suggestion has been made that the intake of calories might also influence the incidence of human cancer Tannenbaum (194) has made a survey of insurance statistics and has suggested that cancer mortality increases with increasing body weight Reasoning from experience with animals, it would appear that the avoidance of overweight might prevent or delay the inception of a tumor but would probably be of no value once the tumor has developed That any conclusion at all should emerge from insurance statistics is the more significant when one recalls that the analyses of human statistics cannot have the accuracy of animal investigations in which pure strains of animals of known genetic composition, living in the same laboratory, are used, and where age, temperature, weight, diet and factors of carcinogenesis can be controlled Furthermore, in an experimental investigation on animals, one may study a single type of tumor, in contrast to some statistical studies on man, in which all types of cancers are grouped together

The effect of caloric reduction on carcinogenesis is so pronounced that this effect must be considered in interpreting any experiment involving the influence of secondary factors on tumor development Thus, it is possible that the retardation of tumor formation by some agent may well be the indirect result of caloric restriction rather than the direct consequence of the therapy itself

An indirect way of achieving caloric restriction in a mouse is to force it to perform physical work When mice bearing transplantable neoplasms were subjected daily to periods of forced exercise, the rate of tumor growth was slower than that found in mice not treated in this manner (180) Conversely, the augmentation of tar cancer in rabbits following injections of glucose (172) might also be a caloric one

*Fat effect* There is abundant evidence that diets high in fat tend to increase the rate of formation of certain types of induced tumors Watson and Mellanby (213) observed that the carcinogenic activity of tar was increased if the experimental mice were maintained on a diet to which 12 5 to 25 per cent butter had been added 55 per cent of the mice fed the butter fat developed tumors as compared to only 28 per cent in the control group Furthermore, 60 per cent of tumor-bearing animals in the butter fed group also had lung nodules compared to 37 per cent with adenomata in the control group (213) Vles, DeCoulon and Ugo (208) noted an augmentation of tar cancer when egg yolk was fed to mice In a series of recent papers, Baumann and his associates studied the tumor-promoting effect of fat in some detail Control mice receiving continuous applica-

tions of hydrocarbon solutions (methylcholanthrene, benzpyrene or dibenzanthracene) required approximately one month longer for 50 per cent of the animals to develop skin tumors than mice treated in the same manner but fed diets to which 15 or 20 per cent of fat had been added (13, 91) Dietary fat also markedly increased the number of tumors in mice treated with an amount of methylcholanthrene that was only sufficient for the production of tumors in a small percentage of the mice (109) In another experiment certain components of fat were investigated in an attempt to identify the active fraction Mice fed 15 per cent of Primex (partially hydrogenated cottonseed oil) had a tumor incidence of 60 per cent, those given 15 per cent of ethyl laurate had a tumor incidence of 63 per cent, in contrast to 11 per cent in the control group Glycerol and the unsaponifiable fraction of the fat were less effective It appeared therefore that the fatty acid fraction was largely responsible for the fat-effect. The highest incidence of tumors appeared when fat was given throughout the experiment, but measurable increases were also observed when fat was fed either during the first two months while the carcinogen was applied or after the application of hydrocarbon had ceased The most effective period was from 2 to 12 weeks after the beginning of the application of hydrocarbon (109) The fatty acids of hydrogenated vegetable oil, prepared free from unsaponifiable matter and resynthesized into triglycerides, had essentially the same tumor promoting activity as natural fat (110) A definite, though lesser effect of fat on the incidence of skin tumors due to benzpyrene was observed in 3 different strains of mice by Tannenbaum (197) Furthermore, the incidence of spontaneous breast carcinoma was significantly increased in DBA virgin mice fed a high fat diet 32 of 50 mice on the high fat diet eventually developed spontaneous breast tumors as compared to only 16 in a similar group fed a control ration Baumann and Rusch (14) demonstrated that diets with a high fat content also accelerated the formation of tumors due to ultraviolet radiation

At least two explanations of the fat-effect have been considered The fat might act locally in the carcinogenic area, by altering the rate of hydrocarbon penetration of the skin or by accelerating the process in some other manner, or it might exert a more generalized systemic influence The local effect is no doubt significant in the dietary experiments because mice fed high fat diets frequently exhibit a marked greasiness of their fur (176, 213) When mice were fed fat as an emulsion in the drinking water, this greasiness was diminished, and the fat consumed appeared to be only one-half as effective in increasing tumor formation as when the fat was mixed with the solid food (110) Lavik and Baumann (110) observed that mice painted with methylcholanthrene consumed 12 to 30 per cent more calories on diets high in fat than on corresponding low fat diets, and a rough correspondence appeared to exist between the caloric intakes of the mice on the various rations and the numbers of tumors that ultimately developed On an equivalent caloric intake, the incidence of tumors in mice on the high and low fat diets was more nearly equal It appeared, therefore, that at least part of the tumor promoting action of fat was due to an accompanying increased consumption of calories (110) Nevertheless, the combined influence of extra calories

and local greasiness may not completely explain the tumor-promoting action of fat, for the incidence of spontaneous breast tumors was increased on high fat diets of controlled caloric intake (197) and for this type of tumor the areas of tumor genesis could not have been influenced by direct contact with fat

To date all natural fats tested appear to augment the incidence of skin tumors due to pure hydrocarbons (13, 91, 109, 110, 197) with the possible exception of wheat germ oil, which has been reported to be without effect in the incidence of skin tumors due to benzpyrene (85) It is probable, however, that this experiment was complicated by a caloric effect since the authors observed a weight loss in the mice receiving the wheat germ oil However, not all types of tumors are affected by dietary fat No effect was observed on the development of sarcomas when benzpyrene, dibenzanthracene or methylcholanthrene were injected subcutaneously into mice or rats (13, 110, 197) and the incidence of the spontaneous lung tumor was likewise unaffected by dietary fat (197)

For still other types of tumors certain fats may actually inhibit carcinogenesis The presence of 5 per cent of hydrogenated coconut oil greatly retards the production of hepatic tumors in rats fed p-dimethylaminoazobenzene (131) On a synthetic diet containing 5 per cent of corn oil, the incidence of hepatic tumors at 6 months ranged from 53 to 64 per cent, but when the corn oil was replaced by hydrogenated coconut oil, the tumor incidence never exceeded 8 per cent while in most groups it was zero Since hydrogenated coconut oil is composed almost entirely of saturated fats, it was thought that the presence of an unsaturated fat might be necessary for the genesis of hepatic tumors However, the inhibiting effect of the hydrogenated coconut oil persisted in rats fed 40 mgm of ethyl linolate daily Other differences between the two oils are in chain length and in the amount of antioxidants present

György and his co-workers (84) have shown that p-dimethylaminoazobenzene was destroyed *in vitro* in diets containing high levels of linoleic acid Obviously if a fat promotes the destruction of the azo dye in the diet, before it gets a chance to act in the critical carcinogenic areas, no tumors will develop, but the protection of the animal would essentially be a reflection of a reduced intake of carcinogen Such an explanation, however, could not explain the protective action of hydrogenated coconut oil since the azo dye was shown to be very stable *in vitro* in the diets fed (131)

At least three other fatty extracts have been reported to reduce the carcinogenic effectiveness of the azo dye when fed with it at levels of 5 per cent of the diet or less rice bran oil (138, 192), an ether extract of yeast (192), and a similar extract of liver (132) "However, the original materials from which these extracts were prepared all happen to be anticarcinogenic by virtue of non-fatty substances such as proteins and B-vitamins which they contain Analyses of rice bran oil for riboflavin and other B-vitamins revealed the presence of many water-soluble materials (192), although in amounts which were probably insufficient to account for the entire anticarcinogenic effect observed" (131)

A second group of fats appears to have no significant effect on the development of hepatic tumors due to p-dimethylaminoazobenzene These include cod liver

oil at 1 or 2 per cent of the diet (132), olive oil at 3 per cent (96, 155), cottonseed oil (132), corn oil (94, 132), butter fat (132), wheat germ oil (191) at 5 per cent, and partially hydrogenated vegetable oil (Crisco) at 20 or 30 per cent of the diet (132). These latter oils, however, were usually fed in experiments designed to reveal the effects of non fatty constituents of the diet, and true differences between the various oils may therefore have been masked. Another complication arises from the fact that as the percentage of fat in the diet increases, the amount of food and therefore of carcinogen consumed by the animal decreases.

When lecithin was fed to rats that were also receiving o-aminoazotoluene, the production of liver tumors was accelerated (4). Apparently this is the only lipoidal substance reported to augment the development of liver cancers. Cholesterol was without effect (4).

The relationship of cholesterol to tumor development has received the attention of a considerable number of investigators, and the literature has been recently summarized (15). Roffo (167) considers cholesterol of prime importance in the development of tumors due to ultraviolet radiation and has stressed the fact that cholesterol accumulates in certain precancerous lesions in rats and in man. The addition of cholesterol to the diet has been reported to hasten the formation of tar cancer in mice (72). However, there is considerable doubt that cholesterol can be regarded as a universal accelerator of the carcinogenic process. When added to the diet it failed to stimulate the production of tumors by ultraviolet radiation in the rabbit or mouse, Shope virus papillomas developed at essentially the same rate in the controls and in rabbits in which a cholesterolemia had been produced, and the addition of 2 per cent cholesterol to the diet of mice failed to alter the rate of formation, or the number of hepatomas induced by o aminoazotoluene (4, 13, 15).

Various fats have been reported to have carcinogenic properties when included in the diet. Roffo (168–171) reported that he had induced adenocarcinomas and sarcomas in the stomachs and intestines of rats fed fats or cholesterol heated to 300°C. The minimum period for the production of these lesions was 16 months. Beck and Peacock (18), however, found no lesions of the glandular stomach in rats that had been fed heated cooking fats and oils for a period of 436 days, although papillomas were frequently present. Since the rats fed the heated oils also developed the clinical signs of avitaminosis A, it was concluded that heated fats contained some substance that interfered with vitamin A metabolism. Kirby (98) heated cholesterol at 270 to 300°C. for half an hour in the air, and fed 20 mgm. of the product daily to rats for a period of two years. No significant lesions from the point of view of carcinogenesis were observed in either part of the stomach. Domagk (67) noted tumors or glandular hyperplasia in a few mice that had received a diet of rice plus 20 per cent of olive oil for a year. In one mouse a gastric adenocarcinoma was found and in others the gastric mucosa was the seat of an extensive polypoid overgrowth which probably was precancerous.

Rowntree, Steinberg, Dorrance and Ciccone (175) reported that a large percentage of rats fed a crude wheat germ oil developed sarcomas arising from various areas of the peritoneum. Most of the tumors appeared within 60 to 200

days after the start of the experiment. Several other investigators attempted to confirm these findings but were uniformly unsuccessful (39, 46, 47, 66, 73, 86, 177).

*Proteins and amino acids.* The importance of cystine for the genesis of several types of neoplastic growths has been stressed by White. Female mice of the $C_3H$ strain were divided into two groups at the time of weaning; one was placed on a diet relatively low in cystine and in total protein while the other group received 0.5 per cent of cystine in addition. The mice on the low cystine diet failed to develop spontaneous mammary tumors, even after 22 months, whereas 97 per cent of the mice on the "high" cystine diet developed tumors (216). Furthermore, the incidence of leukemia induced by methylcholanthrene in mice of the dilute brown strain was reduced when the concentration of cystine in the diet was decreased to such a level that weight increases were prevented. Only 17 per cent of the mice on the low cystine diet developed leukemia as compared to 92 per cent of the controls on a high level of cystine (218). White and Edwards (217) also demonstrated that cystine or methionine could influence the production of hepatic tumors in rats. When rats were fed a low cystine basal diet containing 0.06 per cent p-dimethylaminoazobenzene, 60 per cent developed liver tumors, as compared to 96 per cent in the animals receiving the same diet plus 0.5 per cent of cystine or methionine. White has also shown that the growth rate of a rat can be retarded by the oral administration of methylcholanthrene, benzpyrene or p-dimethylaminoazobenzene (215, 219). This effect was observed only on diets containing high amounts of fat and a borderline level of protein. Growth was restored even in the presence of the carcinogen when l-cystine or dl-methionine were added to the diet. It was suggested that the carcinogens increase the demand on the animal for a sulfur containing amino acid needed for detoxication, and as a result, the synthesis of new tissue protein is prevented (215).

György, Poling, and Goldblatt (83) reported that the combination of cystine and choline retarded liver cirrhosis and hepatomas in rats fed a semi-synthetic diet. This finding however was not confirmed by White (217), and Mori (136) found that 1 gram of cystine per kilo of diet did not inhibit the genesis of liver tumors in rats fed a diet of rice and carrots.

Under certain conditions the development of hepatic tumors induced in rats by the ingestion of p-dimethylaminoazobenzene can be retarded by increasing the amount of protein fed. Kensler and his associates (94) observed that neither casein alone nor riboflavin alone prevented tumor formation, but that the two factors together were very effective. Nakahara and his collaborators (155) reported that 10 per cent of fish protein added to a diet of rice and carrots failed to reduce the incidence of tumors due to the azo dye. Sugiura and Rhoads (192) observed no protection when casein was added to the rice-carrot diet although the nutritional state of their animals was markedly improved by the addition. When suitable B vitamins were given in crystalline form, the incidence of hepatic tumors was essentially the same whether 12 or 18 per cent of casein was fed (133).

*Vitamins* In studying the tissue changes that developed in rats as a consequence of vitamin A deficiency Wolbach and Howe (221) were impressed with the greatly augmented growth activity of epithelium In some animals the number of mitotic figures in the replacing epithelium and the response of the connective tissue and of blood vessels were such as to suggest "the acquisition of neoplastic properties" Other investigators have also observed that a chronic vitamin A deficiency led to changes in some tissues which might be described as preneoplastic Orton, Burn and Smith (160) noted odontomas on the teeth of rats surviving over a year on a diet low in vitamin A and in a few instances, supernumerary incisor teeth were seen These odontomas arose from the pulp and consisted chiefly of spindle cells similar to the embryonic cells of the pulp tissue and apparently were not growing very actively Proliferative stomach lesions have been reported in rats on a diet deficient in vitamin A (78) but such pathological changes can in no way be considered specific since many dietary deficiencies have been shown to induce similar changes (100, 140)

These observations raised the question as to whether a deficiency of vitamin A in the diet would promote the genesis of tumors by a carcinogenic hydrocarbon To explore this possibility Howe, Elliott and Shear (90) placed one half of a series of rats on a diet rich in vitamin A while the remainder were given a diet deficient in this vitamin Cholesterol pellets containing 5 per cent of benzpyrene were implanted subcutaneously in all of the animals If the diet was markedly low in vitamin A the rats died during the latent period of tumor production but when enough carotene was added to permit the rats to survive the latent period, there was no indication of a significant difference in response to this carcinogen

Of the various members of the B-complex only riboflavin, pyridoxine and biotin have shown any significant influence on tumor genesis. Riboflavin added to the diets of rats fed p-dimethylaminoazobenzene inhibited the formation of hepatomas (94, 132, 133) When a diet containing rice and carrots was fed, the protective action of riboflavin was observed only in combination with a high protein level (94) With synthetic diets, however, riboflavin alone at a level of 10 mgm per kilogram of ration completely prevented tumor formation up to 6 months, whereas no protection was noted with 0 5 mgm per kilogram of diet (133) Although riboflavin has a pronounced effect on the prevention of hepatomas in rats fed p-dimethylaminoazobenzene, high levels added to the diet did not retard the growth of established spontaneous mammary tumors in mice, or of the hepatoma 31 transplant in rats In fact, the growth rate of these tumors was decreased in animals on a riboflavin deficient diet (141) Riboflavin did not prevent the formation of skin tumors due to methylcholanthrene (110)

The incidence of hepatomas induced in rats by p-dimethylaminoazobenzene was greatly decreased when the level of pyridoxine in the diet was reduced to 0 2 mgm or less per kilogram of ration (133) When pyridoxine was low or omitted entirely from a synthetic ration, less than 7 per cent of the animals developed liver tumors, as compared to an incidence of over 50 per cent on the control diets containing 2 5 mgm of this vitamin per kilogram A pyridoxine deficiency has also been shown to retard tumor formation in mice treated with a

solution of 0 2 per cent methylcholanthrene (102) In contrast to the results with epithelial tumors in mice and hepatomas in rats, no very pronounced effects of pyridoxine deficiency were observed on the development of sarcomas induced by the subcutaneous injection of methylcholanthrene (102) It is of interest, however, that the growth of transplantable tumors in both rats and mice is retarded by a diet deficient in pyridoxine (31, 102)

The retarding effect of diets deficient in pyridoxine was independent of caloric intake In fact, in one experiment in which rats received p-dimethylaminoazobenzene and a low level of pyridoxine, the average daily food consumption was higher and the animals maintained their weights better than in the control group (133) Adult rats can maintain their weights for long periods of time in the absence of dietary pyridoxine (130), but the addition of p-dimethylaminoazobenzene to the diet caused a loss in weight (133)

DuVigneaud and his collaborators (71) have reported that biotin increased the incidence of liver cancers induced by feeding p-dimethylaminoazobenzene This has been confirmed in part by the observation that egg white in the diet suppressed the formation of liver cancer in rats fed p-dimethylaminoazobenzene (103) Egg white contains an abundant amount of avidin, a substance that renders biotin unavailable as a food factor West and Woglom (214) did not observe any retardation in the growth of a transplantable tumor in mice fed avidin concentrates However, these latter workers studied the effect of avidin on the growth of established tumors while the investigations of DuVigneaud (71) and those of Kline et al (103) were made on the genesis of neoplasms

Maisin, Pourbaix and Cameran (125) examined the action of riboflavin, vitamin $B_1$, and nicotinic acid fed to mice on the production of tumors by the application of benzpyrene to the skin The addition of sodium pyrophosphate plus 20 micrograms of vitamin $B_1$ daily to the diet of each mouse had no effect on the appearance of papillomas but did delay slightly the malignant change of these tumors On the other hand, when mice were treated with methylcholanthrene and fed diets containing either an abundant or a poor supply of vitamin $B_1$, no difference in the rate of tumor development between the two groups was observed (82)

Adamstone (1) fed ferric chloride to developing chicks in order to destroy the vitamin E content in the food A large proportion of the chicks on this ration developed lymphoblastomata involving the heart, lungs, liver, spleen, pancreas and gizzard It should be remembered that leukemia in chickens is rather common

*Miscellaneous diets* Various animal tissues are also known to influence carcinogenesis Maisin and his associates (122) have shown that the inclusion of liver, pancreas or intestinal mucosa in the diet of tarred mice promoted cancer growth whereas brain, thymus, bone marrow, dried gastric mucosa or dried lymph nodes inhibited the development of cancer induced by tar Growth-inhibiting and growth-promoting factors were often found in the same organ and, in general, substances which augment tumor genesis were water soluble at pH 7–8 4 and were relatively insoluble in ether, whereas the inhibiting fractions are soluble in

other and are relatively insoluble in acetone (122) An acceleration of skin tumors following applications of tar or benzpyrene was observed in mice that received whole liver in their diets (13, 211) However, the feeding of liver did not augment the formation of tumors due to ultraviolet radiation or to the injection of hydrocarbons (13, 137)

The most striking effect of dietary liver is observed on the development of liver tumors induced by the azo dyes When 10 per cent dried whole liver is included in the diet of rats fed or injected intraperitoneally with p-dimethylaminoazobenzene, the formation of liver cancer is greatly retarded (132, 135, 137, 154, 155) An alcohol liver fraction, i e , that portion of the water soluble material of whole liver that is insoluble in 70 per cent alcohol gave good inhibition when fed at a level of 2 per cent (132) In a search for other animal tissues which might likewise inhibit the formation of liver cancer, Mori (135) found that kidney was also effective but spleen, muscle, brain, lung, stomach, intestine, testicle and bile had no effect The pancreas even stimulated tumor formation to a mild degree. Apparently the retarding effect of liver is limited to the genesis of hepatomas since liver feeding did not protect against the development of sarcomas in strain C mice induced by the subcutaneous injection of o aminoazotoluene (203)

An antiblastogenic factor has been found in fresh beef heart The incidence of tumors in mice painted with a solution of methylcholanthrene was reduced from 42 per cent in the controls to 27 per cent in the group that were fed fresh beef heart Drying the heart tissue 24 hours at 70 to 90° destroyed the factors responsible for the inhibition (123)

The retarding influence of yeast on the genesis of tumors has also been reported Nakahara, Fujiwara and Mori (153) observed a reduction in the number of hepatomas in rats given weekly intraperitoneal injections of p-dimethylaminoazobenzene and fed dried baker's yeast to the amount of 15 per cent Between the 257th and 279th day liver cancer was found in 3 of the 6 survivors of the control group as compared to no tumors in 10 survivors of the rats fed yeast Other investigators (132, 193) have also shown that hepatomas induced in rats by the ingestion of p-dimethylaminoazobenzene could be inhibited by including at least 15 per cent yeast in the diet Yeast heated to 80°C for 7 minutes was found to decrease the incidence of skin tumors in mice due to benzpyrene (124)

A decrease in the rate of formation of hepatomas due to azo dyes has been noted when rats were fed various grain diets Thus when wheat (9, 77, 97, 206), rye (120) or millet (139) was used as a basal diet instead of rice, the incidence of liver tumors was reduced On the other hand, a water soluble rice bran concentrate has been shown to augment the genesis of liver tumors induced by p-dimethylaminoazobenzene (132) This concentrate is an excellent source of the known vitamins of the B complex with the exception of riboflavin It should be recalled that synthetic diets low in riboflavin but with an abundant supply of the other vitamins of the B-complex have been shown to augment the formation of liver tumors (133)

B *Miscellaneous* The influence of various hormones on tumor genesis has

been the subject of a considerable number of investigations and most of these have been concerned with the effect of the sex hormones This information has been summarized in previous reviews (3, 81, 106, 115, 142)

It is generally accepted that in experimental mice the tendency to develop spontaneous mammary tumors is controlled by inheritance, hormonal stimulation and by a third factor which is usually transferred by nursing The significance of heredity in this phenomenon has been summarized by Loeb (114) Recently the importance of the factor transmitted by the milk has been clearly demonstrated and the results of the investigations have been summarized by Bittner (34) There is now some evidence that the agent responsible for this effect is a protein of a size comparable to that observed for some of the viruses, and the suggestion has been made that the factor might indeed be a virus (33) The agent has also been demonstrated in the spleen, blood, and lactating mammary tissues as well as in the milk (8, 222), and there is some evidence that it might also exert an effect on embryos *in utero* (74) Furthermore, it has been reported that the growth of some types of mouse leukemia was also influenced by foster nursing although this was not true for all strains (99, 112)

Turner (202) noted that a growth-inhibiting substance derived from human urine prevented, to a limited degree, the formation of tumors in mice following the injection of dibenzanthracene or methylcholanthrene Of 54 mice injected with either of these hydrocarbons, followed by daily injections of the inhibitor, 30 per cent developed tumors as compared to 80 per cent of the controls not treated with the extract The urinary derivative had little or no effect on transplanted or spontaneous tumors in mice

In a number of papers Maisin and his collaborators (104, 126) have reported that certain organic peroxides altered cancer incidence in mice treated with benzpyrene Since tumor tissue has a greater anaerobic glycolysis than corresponding normal tissues, it was thought that the use of substances which readily yield free oxygen might retard tumor genesis These investigators injected these "peroxides" either in aqueous solution or in the form of crystals, and reported that there was a different effect on cancer incidence depending upon the concentrations used Strong concentrations, i e , up to $1 \times 10^{-3}$, had an activating effect, hastening the appearance and increasing the incidence of malignant growths Beginning with a dilution of $1 \times 10^{-5}$ an inhibitory effect was observed and this inhibition increased up to a dilution of $1 \times 10^{-18}$ In a comprehensive series of experiments Belkin (20–22) was unable to confirm these results The latter investigator tested the effect of the peroxide on the incidence and growth of transplantable, induced, and spontaneous mouse tumors, using a very large number of animals in his series The results were uniformly negative in all cases

## SUMMARY

The extrinsic factors that alter the action of carcinogenic agents include various chemical and physical agents such as fats, oils, "irritating" substances, trauma and radiant energy all of which have a pronounced influence on tumor

formation when applied directly to the tissues undergoing carcinogenic changes Other factors are active when administered at a distance from the tissues undergoing neoplastic conversion These include alterations in the diet involving calories, fats, proteins and amino acids, vitamins, and modifications in the balance of certain hormones

It should be stressed that altering the carcinogenic process does not necessarily imply a similar effect on the growth of tumors The investigations cited illustrate the extreme caution necessary in the interpretation of results observed Factors that influence tumor growth might be obscured when overwhelming doses of carcinogen are employed, and any treatment that interferes with the dietary intake or with the general health of the animal may lead to false conclusions

The ultimate aim of experiments of this nature is a better understanding of the mechanism of neoplastic development Minor successes have been attained in this direction, and the suggestion has been made that carcinogenesis progresses not as a single process but rather in two or more distinct stages *a*, the preneoplastic stage or the latent period of carcinogenesis, and *b*, the neoplastic stage Various agents have been shown to act on one or the other period In general, however, the experiments raise more questions than they answer Thus, the nature of the effect due to fat, to croton resin, to calories, or to the factors involved following injury, as well as the various effects of dietary constituents all must await further experimentation

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# DISTRIBUTION OF VITAMIN A IN TISSUE AS VISUALIZED BY FLUORESCENCE MICROSCOPY

HANS POPPER

*The Hektoen Institute for Medical Research of the Cook County Hospital and the Departments of Pathology and Physiology of the University of Illinois, College of Medicine, Chicago Illinois*

Attempts to localize biologically active substances in tissues by histo-chemical and histo-physical methods have been carried out repeatedly in order to clarify their rôle in the organism (1, 2) The demonstration of vitamin A by a histologic method seems to be important in view of its still problematic rôle in the body

Vitamin A lends itself to morphologic demonstration because its fat solubility prevents its solution in aqueous fixatives There have been some attempts to visualize it by histo-chemical methods, but these were of questionable specificity (3, 4, 5) In recent years the fluoresence of vitamin A in ultra violet light has been used for its demonstration in tissues This review presents the findings obtained by this method

Historical introduction In 1932, Von Querner (6) described a luminescent substance (Leuchtstoff X) in isotropic fat droplets in the liver cells Its strong fluorescence was quickly destroyed by the ultra violet light eliciting this phenomenon In further studies he (7) found this substance soluble in lipoid solvents and not in aqueous solutions After various attempts to identify it by histo-chemical examinations, he (8) finally concluded that it is related to pigments, probably the fat soluble carotenoids Since Querner encountered a similar fluorescence in fish liver oils and in vitamin A concentrates, he assumed that the luminescent substance found in liver, retina, adrenal cortex and pituitary body is vitamin A

Querner deserves the credit for having pointed to the vitamin A fluorescence as a means of demonstrating vitamin A in tissues even though his histological descriptions were incorrect and the few animal experiments reported were insufficiently controlled. Prior to Querner, Hirt (9) described bright yellow fluorescent droplets in the wall of the liver sinusoids in living animals which he (10) later related to vitamin A. Querner localized the fluorescence in the lipoid droplets of the epithelial liver cells, whereas Hirt and his co-workers found them in the Kupffer cells In a more recent, chiefly vital, microscopic study Hirt and Wimmer (11) found the greatest amount of vitamin A fluorescence in the Kupffer cells of the liver and endothelial cells of the lung, less in the endothelial cells of other organs The parenchymatous cells were reported as free of vitamin A fluorescence In addition, they found in living organs different fluorescent substances which they associated with various members of the vitamin B complex and different combinations of them with proteins, with vitamin C, or with the active liver principle of pernicious anemia Their reports, so far accessable, do not give sufficient information as to how these different fluorescent details in the living organs may be differentiated and fail to offer con

trolled studies as to the specificity of the fluorescent phenomena As long as there is not more known about the basis for their assumption, Ellinger's (12) criticism against their theories appears justified Nevertheless, their observations in living organs are important for the evaluation of the somewhat clearer pictures of fixed specimens Far better substantiated appear the observations of Jancsó and Jancsó (13) who observed an increase of the characteristic fluorescence of the liver following the administration of carotene and vitamin A, and also a green fading fluorescence in the pigment layer of the retina, depending on the degree of light adaptation The recent German investigations of Schairer, Rechenberger, Gockel and Patzelt (14), Patzelt and Schairer (15), and Schairer and Patzelt (16) are accurate and well controlled studies of vitamin A fluorescence Their observations appeared simultaneously with those of our group in this country Since the studies were obviously independent they confirmed each other Their reports and the more extensive ones of our own group represent the basis of the following presentation

A METHOD OF HISTOLOGICAL DETERMINATION OF VITAMIN A *1 Physical Principle* Vitamin A has a maximum absorption in the higher ultra-violet range at 328m$\mu$ (17) If exposed to ultra-violet light of similar wave length, vitamin A assumes a bright green, rather quickly fading fluorescence (18, 19) With given solvent and illumination, the fluorescence is the brighter and the fading the slower the higher the concentration of vitamin A If the illumination is reduced, *e g*, by screening out some of the light, the fluorescence is duller, but the fading protracted Similarly, in certain solvents equal amounts of vitamin A fluoresce more brightly and fade faster than in others (20)

*2 Principle of Fluorescence Microscopy* The visible picture is observed which is produced if ultra-violet rays strike fluorescent structures in the examined specimen These structures change the ultra-violet light which they absorb to visible light of different wave length The remaining ultra-violet rays are absorbed by a filter in the eye piece (*e g*, Leitz ultra-violet protecting filter no 8574-A)

Various applications of fluorescence microscopy in technical and biological sciences have been reported (21, 22, 12) In biology, either living objects, usually in incidental light, (vital-microscopy, see Ellinger 12, Singer 23) or specimens, usually in transmitted light, have been studied The fluorescence microscopic picture of human organs has been described in detail (Hamperl 24 Sutro 25, and others) Furthermore, staining with fluorescent dyes (fluorochromy) has been applied (22)

*3 Fixing* Fixation in solution of formaldehyde U S P 1.10, up to eight hours in animal organs, up to eighteen hours in human organs, does not affect the vitamin A fluorescence The sections are mounted in water and examined immediately (14, 26) The common mounting media for frozen sections either yield a disturbing fluorescence (glycerine) or dissolve vitamin A (mineral oil)

*4 Microscope* The light of a mercury vapor bulb is filtered through an ultra-violet filter (such as the Corning glass color filter no 584) to eliminate the visible light The residue of red rays is absorbed by a glass cell containing

a 5 per cent cupric sulfate solution or an additional Corning glass color filter no 428 Since the vitamin A fluorescence in tissue sections is produced by rays in the longer ranges of the ultra violet which are not absorbed by glass, a standard microscope with a standard Abbe condenser is used (27, 15)

*5 Observation* Under the fluorescence microscope a dark field is seen in which fluorescent material stands out Nearly all tissue constituents reveal some kind of fluorescence This permits orientation in an unstained tissue section Vitamin A fluorescence is considered any fading fluorescence of green hue in contrast to other types of green fluorescence which are ultra violet stable

*6 Localization of the Fluorescence* In frozen sections stained with hematoxylin or methylene blue the vitamin A fluorescence is visible (7, 26) If the ultraviolet filter is changed to a ground glass filter the sections can be studied in visible light and the fluorescence localized. For the study of fat distribution, sections were examined after treatment for three minutes with a 0 1 per cent aqueous phosphin 3R solution By this method fats reveal a silver white fluorescence on a brown background. This procedure permits more and finer droplets to become visible than is possible with the routine fat methods, because of the water solubility of the dye (27)

*7 Photography* Vitamin A fluorescence is photographed by the use of a 35 mm camera with a periscope view finder on the microscope attachment The film material should be either of maximal daylight sensitivity or sensitized for green and yellow (28)

EVIDENCE FOR THE SPECIFICITY OF THE VITAMIN A FLUORESCENCE Several points of evidence which are partly suggestive and partly conclusive, support the belief that the green, quickly fading fluorescence in tissue sections is due to the presence of vitamin A

*1 Suggestive Evidence (as to the fluorescence in human and animal organs)* A *Visual appearance of the fluorescence* The green fading fluorescence of adrenal and liver sections stimulates under the microscope the fluorescence of droplets of an oil in water emulsion of oils containing vitamin A, such as percomorph or halibut liver oils (8), various commercially available vitamin A concentrates or solutions in corn oil of vitamin A alcohol, vitamin A acetate or vitamin A palmitate (27) Attempts to supplement the visual observation by spectroscopic analysis of the green fluorescence in tissue sections, by Querner (8), Schairer and his co-workers (14) and ourselves, did not succeed because of the quick fading of the fluorescence However, if the light which excites the fluorescence, is roughly analyzed by the use of glass filters, the rays producing the green, fading fluorescence in tissues are localized between 310 and 350 mμ, that is, in the neighborhood of 328 mμ, the specific absorption maximum of vitamin A

Carotene imparts under the microscope a dull, very slowly fading green fluorescence which is visible only in higher concentrations and which is easily differentiated from the vitamin A fluorescence (14, 26)

The biologically inactive anhydro ("cyclized") vitamin A (29, 17) gives chemical reactions similar to those of vitamin A, but imparts not the charac

teristic green, quickly fading fluorescence of vitamin A, but a dark brown fluorescence which slowly changes to a duller green and then slowly fades

Corn oil and cotton seed oil do not show vitamin A fluorescence The following fat soluble biologic substances dissolved in corn oil as tested under the fluorescence microscope in an oil-in-water emulsion do not show a green, quickly fading fluorescence Several vitamin D preparations, alpha- and mixed tocopherol, synthetic vitamin K (2 methyl-3-phytyl-1-4 napthoquinone), estrone, alpha-estradiol, testosterone, progesterone, desoxycorticosterone, cholesterol, cholesterol esters and 1,2,5,6 dibenzanthracene

B *Histo-chemical analysis* The green, fading fluorescence in the tissue sections is abolished by reagents which either dissolve or destroy vitamin A and is not changed by reagents which have no influence upon vitamin A (7, 14, 26)

C *The distribution of vitamin A fluorescence* Its distribution in animal or human organs agrees with that found by chemical analysis using the antimony trichloride (Carr-Price, 30) reaction, by spectroscopic analysis, and by biological assay as far as they are available (31, 32,33, 34)

D *The relation of the characteristic fluorescence to the vitamin A metabolism in human beings* In a large series of human livers obtained at biopsy or autopsy, a fairly good agreement has been found between the amount of vitamin A fluorescence and the concentration of vitamin A as determined chemically (35), this partly in contrast to the findings of Schairer *et al* (14) who considered the existence of a type of vitamin A, which is chemically demonstrable, but not fluorescent in tissues Experience permits one to predict the result of the chemical analysis on the basis of the histologic picture In diseases associated with prolonged malnutrition or in other conditions in which the chemical analysis yielded traces of vitamin A, usually very small amounts of vitamin A fluorescence were found (27) After feeding of high doses of vitamin A before operations, very high amounts of vitamin A have been found in the biopsy specimens obtained during the operation (36, 35)

E *The characteristic fluorescence in deficiencies other than vitamin A deficiency* In rats deficient in vitamin $B_1$, $B_2$ and D, as well as in guinea pigs deficient in vitamin C, a normal distribution of the vitamin A fluorescence was seen (37)

*2 Conclusive Evidence as to the Fluorescence in Organs of Rats* A *Depletion experiments* While rats are kept on a vitamin A deficient diet, the green, fading fluorescence in liver, adrenal, ovary and lung gradually disappears (14, 15) parallel with a decrease of the liver vitamin A as demonstrated chemically (38, 39) The fatty deposits in the livers of vitamin A deficient rats intoxicated with carbon tetrachloride did not show vitamin A fluorescence fat appearing in the liver reveals the green, fading fluorescence only if vitamin A is available (40)

B *Repletion experiments* If vitamin A is fed to rats depleted of vitamin A, a green, fading fluorescence reappears in typical distribution (14, 15) and the amount of this fluorescence found agrees with the amount of vitamin A fed and with the amount found chemically in the liver (38) If much vitamin A is fed over a prolonged period, parallel with an extreme increase of the vitamin A concentration, a very striking vitamin A fluorescence appears in organs normally

showing moderate fluorescence and some in organs normally free of vitamin A (11, 13, 14, 38, 39) If carotene is fed, after a somewhat longer interval vitamin A fluorescence reappears (15, 38), again parallel to the amounts of carotene given and to the vitamin A content of the organ. Feeding of other substances such as different oils or vitamins failed to produce the characteristic green fluorescence in the organs of vitamin A deficient rats (38)

It was important to rule out the possibility that the green, fading fluorescence in tissue sections is due to riboflavin which, dissolved in the aqueous phase of a water in oil emulsion, shows such a fluorescence Indeed, Hirt and Wimmer (41, 11) considered a part of the green, fading fluorescence seen in living tissues to be due to vitamin $B_2$ However, the green, fading fluorescence in fixed tissues is dependent upon the alimentary intake of vitamin A and independent of the intake of $B_2$, and is not affected by reducing agents (14, 38) Possibly, the water soluble riboflavin disappears from the tissues during fixation and preparation of the sections (38)

Summarizing, conclusive evidence exists that the green, fading fluorescence in sections of rat organs is due to vitamin A. For human organs only suggestive evidence is available Consequently, in describing the green, fading fluorescence in rats one is justified in calling it vitamin A. In other animals or in human organs, one should speak of vitamin A fluorescence, which indicates that it can be caused by vitamin A but does not exclude other, so far unknown, substances

Stability of the vitamin A fluorescence Since vitamin A is quickly destroyed by exposure to air or oxidizing agents (42) the vitamin A fluorescence disappears from frozen sections within a few hours The speed of disappearance is greater in animal than human organs with variations between different organs It vanishes much faster from the Kupffer cells than from the epithelial cells of the liver, although the fat distribution remains unchanged It disappears much faster from tissue sections kept in water than from those kept in plasma, serum, or liver emulsion. This indicates the presence of a protective principle This principle which in smaller amounts is found in urine, bile and organ emulsions is thermostable, not ether soluble and is more effective at 37° than at room temperature Its biologic significance is still problematic (43)

Roentgen irradiation does not decrease the vitamin A fluorescence (44), contrary to what Querner (45) reported

Influence of carrier substances on vitamin A fluorescence As in non biological solvents, the vitamin A fluorescence in tissue varies in character, depending on the carrier for vitamin A. In larger fat droplets it is usually less bright, but more slowly fading than in smaller ones Another factor is the presence of pigments which screen the ultra violet rays out, thus reducing the brightness, but retarding the fading of the fluorescence The presence of carotene in the human liver explains why the fluorescence is more slowly fading but duller than carotene-free rat livers. These variations in brightness with equal amounts of vitamin A prevent an exact quantitative determination of vitamin A in tissues by fluorescence microscopy

*Relation Between Fluorescence and Vitamin A Concentration* Vitamin A

fluorescence is visible in droplets of an oil-in-water emulsion if the oil contains more than 20 micrograms of vitamin A per gram, whereas the dull carotene fluorescence is revealed by oils containing more than 150 micrograms per gram The vitamin A concentration of the individual fluorescent detail in the tissue is above this level In liver, containing more than 1 5 micrograms of vitamin A per gram, vitamin A fluorescence is encountered (38) In organs in which only certain structures contain vitamin A, the histological method is far superior to the chemical analysis

VITAMIN A DISTRIBUTION IN DIFFERENT ORGANS UNDER NORMAL AND PATHOLOGICAL CONDITIONS *Liver* The liver is the chief depot of the body for vitamin A (31, 32) In the livers of human beings, rats, rabbits, monkeys, dogs, guinea pigs and frogs, vitamin A fluorescence has been described in several sites (14, 26, 38, 46)

I *In the Kupffer cells* The fine fat droplets which normally fill the cytoplasm of the Kupffer cells (47) reveal vitamin A fluorescence, except in nutritional deficiency (38) Their absence is uncommon but not necessarily pathologic

II *In the epithelial cells* (a) Fine fat droplets occur near the borders adjacent to the sinusoids These droplets, demonstrated with sensitive fat stains, are arranged like a string of beads If large amounts of vitamin A are present those droplets reveal marked fluorescence They may enlarge and then several of them fill the cytoplasm (b) Large fat drops, demonstrated by routine fat stains One of them fills the cytoplasm and usually pushes the nucleus to the side Their vitamin A fluorescence is very variable and usually lower than that of the small droplets, even in livers containing much vitamin A (c) Fine bars or droplets, irregularly aggregated throughout the cytoplasm and only sometimes lining up on the edge Their quickly fading fluorescence is hardly recognized under low magnification, they are not demonstrable by phosphin 3R Their appearance simulates that of mitochondria (48) That mitochondria contain vitamin A has been proven chemically (49) and suggested by histo-chemical studies (4, 5). Mitochondria of germ cells contain also carotenoid pigments (50) (d) Lipofuscin (wear and tear pigment), which has been considered as a protein compound colored by carotene (51) If the granules show fat reaction and vitamin A fluorescence their red-brown fluorescence appears only after the latter has faded (e) The cytoplasm imparts a diffuse green, fading fluorescence which is removed by alcohol It seems to be due to small amounts of vitamin A diffusely spread over the cytoplasm

The different sites of vitamin A fluorescence in the liver cause a great variability of the picture with and without relation to the lobular topography The significance of this polymorphic distribution is as yet unexplained and only a few points can be gathered from experiments on animals under controlled conditions

I *Influence of Various Experimental Conditions Upon the Vitamin A Distribution in the Rat Liver* A *Influence of diet* 1 *Hypervitaminosis A* Livers of rats, which had received large doses of vitamin A for several weeks and showed the signs of hypervitaminosis A (52, 53, 54, 55), reveal an extremely strong

vitamin A fluorescence The enlarged Kupffer cells are very strongly fluorescent and rich in fat (54, 56), whereas the epithelial cells show the usual fluorescence, chiefly imparted by fine fat droplets and also by the cytoplasm. Around the periportal and central veins, fine fluorescent fat droplets appear in endothelial cells and other connective tissue cells (38, 39)

II *Unbalanced Nutrition* Rats kept for more than three weeks on otherwise balanced diets consisting predominantly of protein, carbohydrate or fat respectively (see 57), reveal characteristic variations partly paralleling that of the fat distribution (58) The rats on a high protein diet show less vitamin A than the others, much of it localized in Kupffer cells, in the lobular centers many mitochondria are seen in the epithelial cells, whereas in the periphery fine droplets line their edge In the animals fed much fat, most of the vitamin A is localized in medium-sized fat droplets on the edge of the liver cells, less in Kupffer cells and some in isolated large fat droplets on the periphery In those given much carbohydrate the most vitamin A, but also the most morphologically demonstrable fat (59), was seen, the strongly fluorescent fat droplets near the border of the cells increased in size towards the periphery of the lobule

In starving rats, the chemical vitamin A concentration does not significantly change (60) despite marked microscopic alterations in fluorescence The vitamin A fluorescence becomes irregular, and moderately fluorescent large fat droplets accumulate at the lobular periphery When in later stages no fat is morphologically demonstrable, the cytoplasm imparts a strong green, fading fluorescence indicating that the latter carries most of the vitamin A instead of the visible fats (58)

III *Choline Deficiency* In rats on choline deficient diets (supplemented by carotene), in which fatty livers develop (61), the vitamin A fluorescence of the fat droplets in the liver markedly decreases First, irregular foci remain in which Kupffer cells and fat droplets in the surrounding liver cells impart the fluorescence, later, only a few isolated fat droplets reveal some vitamin A (62) If vitamin A instead of carotene is fed, the Kupffer cells maintain vitamin A, whereas the liver cell fat is devoid of it It appears that choline or related substances (labile methyl groups) are connected with the presence of vitamin A in the fat of the liver cells. If additional studies corroborate the impression that in choline deficiency vitamin A storage in the Kupffer cells occurs only after vitamin A and not after carotene feeding, it would indicate that only the liver cells are able to split vitamin A into carotene, something they are unable to do in a fatty liver It has been repeatedly claimed that liver damage interferes with the conversion of carotene into vitamin A (63, 64, 65, 66 and others)

B *Experimental Liver Damage* I *Intoxication with Hepato-Toxic Drugs* Intoxication with drugs with possible hepato-toxic effect, as phosphorus, bile acids, thyroxin, phenolphthalein and sulfanilamide, causes a shift of the vitamin A fluorescence in the liver (40) without necessarily reducing the hepatic vitamin A concentration (63, 67, 68, 69, 70) Within one day after administration of carbon tetrachloride (40) the fluorescent small fat droplets near the border of the liver cells enlarge in the lobular center which leads to an accumulation of

strongly fluorescent medium-sized droplets Following repeated administration, the vitamin A disappears from the Kupffer and liver cells in the uninvolved peripheral area and is seen only in the central fatty areas After more than one month, only around the central area a few large fat droplets and some histiocytes reveal vitamin A fluorescence, whereas fatty areas in the intermediary and even peripheral zone are no longer fluorescent, the chemical concentration in this stage is reduced (see also Haig and Post, 71) If the rats receive high vitamin A supplements, the vitamin A fluorescence in the fatty area is more marked and the Kupffer and liver cells in the uninvolved area retain it longer With a high carbohydrate diet the fatty changes are less marked than with high fat or protein diet and the normal Kupffer and liver cells keep vitamin A longer During recovery from this intoxication, rats on a high protein diet show vitamin A in the Kupffer and liver cells of the uninvolved areas before rats fed much fat or carbohydrate do (faster regenerations)

II *Cirrhosis* In animals with experimental dietary cirrhosis as produced by György and Goldblatt (72), the vitamin A distribution is the more irregular the farther advanced the liver damage In early stages a non-lipid soluble material with non-fading, gold-brown fluorescence is seen in liver cells and the adjacent Kupffer cells Later it accumulates in connective tissue cells of the proliferated periportal spaces (73) This material, which is fluorescent even in paraffin sections, is apparently a break down product of fats It seems identical with ceroid, described by Goldblatt and György (72) and Lillie et al (74)

III *Carcinogens* Some carcinogenic hydrocarbons reduce the vitamin A stores in the liver (75, 76, 77, 78), especially that of the mitochondria (49) However, in the recovery a normal fluorescence microscopic picture is seen (Marron, 79)

C *Depletion of Vitamin A Depots* Rats put on a vitamin A deficient diet immediately after weaning lose their small vitamin A stores within 22 days, before their weight becomes stationary or clinical signs of vitamin A deficiency appear (15, 38) The fluorescence of the cytoplasm disappears first, then that of the fine droplets near the border of the liver cells, and finally that of the Kupffer cells, fat droplets retain it the longest If rats are put on the vitamin A deficient diet a few days after weaning their depletion is retarded (see 80) Adult stock animals need about six months for depletion Albino rats are more quickly depleted than pigmented ones (16)

If rats are first made hypervitaminotic and then put on a vitamin A deficient diet (39) fluorescence of the Kupffer cells decreases first, that of the liver cells remains The marked preponderance of the fluorescence of the Kupffer cells gradually subsides, after the vitamin A concentration drops to a third of that in the hypervitaminotic stage The decrease of the Kupffer and liver cell fluorescence is then parallel In advanced hypovitaminosis droplets are found in the epithelial cells very close to the Kupffer cells and once more a preponderance in the Kupffer cells appears Finally, a few Kupffer cells show the last remnants of fluorescence Male rats are depleted faster than female rats The serum vitamin A, which was higher in males than in females, was not related to the fluorescence These experiments indicate that the Kupffer cells not only

distribute vitamin A but also destroy it They apparently cause the rapid vitamin A loss in hypervitaminotic animals on a vitamin A deficient diet (81) Whether this non-economic utilization is related to a deficiency in vitamin E (82) remains to be examined

D *Repletion of Vitamin A Depot.* After feeding of more than 250 units of vitamin A to rats, the liver of which is free of vitamin A for a few days, the fluorescence reappears (15), first in the Kupffer cells and then in small droplets in the adjacent part of the liver cells The speed of reappearance depends on the amount fed (38) In animals depleted for more than a week the repletion is irregular, apparently due to disturbance of intestinal absorption The results of feeding carotene are variable and the fluorescence appears later and in smaller amount, before the appearance of vitamin A fluorescence, the epithelial cells of the liver reveal a diffuse, faint, green, slowly fading fluorescence, probably due to the temporary presence of carotene (15, 38) For replacement of vitamin A by subcutaneous or intraperitoneal administration, larger doses and a longer interval are required (see 83, 84, and others) Carotene administered parenterally did not restore the vitamin A fluorescence (15, 38) After feeding of vitamin or carotene to rats, intoxicated by carbon tetrachloride, the fluorescence appears in the large fat droplets of the liver and only with large doses in the unaltered areas This indicates a higher avidity of the fatty areas for vitamin A (40)

2 *Variations in the Distribution of Vitamin A Fluorescence in the Human Liver* The marked variations encountered were not caused by postmortal changes since specimens of livers obtained for biopsy during laparotomy reveal similar pictures (36) Certain findings were characteristic even in respect to the marked polymorphism (26, 85, 35, 86)

A *Fat Deposition* Localized or generalized infiltrations of medium-sized and large fat droplets may show absence of, moderate, or strong, vitamin A fluorescence In alcoholics, the picture often resembles that of the choline deficient fat liver (62) This raises the question whether similar dietary factors may not decide the vitamin A fluorescence of the large fat droplets in the human liver

B *Preponderance in Kupffer Cells* In the presence of huge amounts of vitamin A, *e g*, after feeding of large doses, most of the fluorescence is in the Kupffer cells and the liver cells have a normal appearance However, in some conditions, like diabetes mellitus uremia, and obstructive jaundice, the Kupffer cells reveal strong fluorescence, whereas the liver cells are almost devoid of it In visible light these areas show usually a toxic edema Since in diabetes clinical signs of vitamin A deficiency occur (87), possibly the lack of vitamin A in the liver cells with accumulation in the Kupffer cells interferes with its utilization.

C *Focal Parenchymal Damage* The vitamin A fluorescence may either disappear from the area involved as in recent thrombosis of the portal vein or with necrosis of the lobular center, or fluorescent material may accumulate in peculiarly shaped sites as in irregular fat droplets in the liver cells or in bizarre shaped Kupffer cells

D *Hepatitis* The greatest polymorphism occurs in acute hepatitis In

acute yellow atrophy very much vitamin A fluorescence may be imparted by irregular shaped fat droplets in liver cells, all visible fat is fluorescent In subacute atrophy less fluorescence is seen and some livers are almost devoid of it In atrophy with transition into cirrhosis, histiocytes and endothelial cells in the vascular connective tissue may be rich in vitamin A fluorescence (see chemical study of Cox 88)

E *Liver Cirrhosis* In arrested forms, the lobules and regenerated pseudolobules reveal a nearly normal distribution Fatty areas may show much or little vitamin A fluorescence Connective tissue cells in proliferated periportal fields often contain fine fat droplets with fluorescence In progressive cirrhosis, especially with jaundice, only scattered droplets in liver cells or some bizarre shaped Kupffer cells reveal vitamin A fluorescence The decreased fluorescence, often despite high fat content, parallels the low chemical vitamin A concentration (89, 90, 91, 92, 64)

F *Irregular Distribution* In addition to the described alterations others occur which are less easily classified The fluorescence pattern may vary from cell to cell or from cell group to cell group without difference of the histological picture in visible light The irregularity of the vitamin A distribution is not mirrored by the chemical analysis Marked degrees of disturbance of the vitamin A fluorescent pattern are associated with parenchymatous damage upon routine histological analysis or clinical study of the liver function (85) Consequently, milder degrees of this disturbance are considered as initial liver damage not recognized by the common routine histological methods (35, 36) According to this standard and in comparison with normal experimental animals almost no human liver available for examination is entirely normal

G *Age Influence* In the fetus much vitamin A fluorescence is found in liver and Kupffer cells Toward term it markedly decreases and in new born infants only traces are seen In the second month, an increase starts and after the second year the vitamin A distribution approaches that of the adult (26) These findings agree with chemical analyses (93, 94, 95, 96)

H *Estimation of the Total Amount of Vitamin A Fluorescence* The total amount of fluorescence was low, in primary liver disease, in obstructive jaundice, malnutrition resulting from tumor and also in renal conditions, this agrees with chemical analyses (92, 95, 97 and others)

*3 Significance of the Vitamin A Distribution in the Human Liver* A few points can be made so far from the study of vitamin A fluorescence

A *The Rôle of the Kupffer Cells* The rôle of the Kupffer cells in fat (98) and vitamin metabolism (11), especially in that of vitamin A (99), has been stressed The Kupffer cells are the last to be depleted and the first to be repleted They absorb vitamin A from the blood and transmit it to the liver cells, as seen from its presence in the portions of the liver cells adjacent to the Kupffer cells, a block in this transmission is probably responsible for the picture in toxic edema They contain the excess of vitamin A in hypervitaminosis and probably destroy it (46) They store carotene if it is injected in aqueous solution (66) After blockade of the Kupffer cells the storage of vitamin A in the liver is interfered with (100, 68, 101)

B *The Rôle of the Liver Cells* The liver cells are the permanent depots for vitamin A. In liver damage they may be unable to hold it or release it to the blood. From large fat droplets vitamin A is very slowly released (40).

C *Relation Between Plasma Vitamin A Concentration and Distribution, and Amount of Vitamin A Fluorescence* (35) Comparison of the plasma vitamin A level with hepatic vitamin A concentration and fluorescence, in specimens of liver removed for biopsy, over a wide range of the plasma vitamin A level shows a parallelism only if an average of a number of cases is taken. In individual cases no definite relation between plasma level and liver stores exists. High plasma level usually indicates normal or high liver stores, whereas low levels may be associated with high or low liver concentration. The plasma level runs somewhat parallel to the vitamin A fluorescence of the Kupffer cells, less so to that of the epithelial cells. With marked discrepancy between plasma level and hepatic vitamin A concentration the vitamin A pattern is very much disturbed, and liver function is impaired.

D *Vitamin A Shift in the Liver as a Cause of Functional Avitaminosis* The shift of the vitamin A from normal to pathologic sites in the liver may explain the drop of the plasma vitamin A level found in acute liver diseases or in pneumonia (Literature, see 102.) A lack of vitamin A intake cannot explain this phenomenon since months are required to reduce the plasma vitamin A level of healthy adults by a vitamin A deficient diet (103, 104, 105). Apparently, the liver regulates the plasma vitamin A level and that explains the parallelism between liver stores and blood level in healthy animals (106, 107, 108). In liver damage in which vitamin A has shifted to places where it is not available for immediate release, the plasma vitamin A level may drop in the presence of quantitatively sufficient liver stores and a functional avitaminosis exist due to disturbed release (109, 97, 35). In healthy young rats clinical signs of vitamin A deficiency appear only a long time after vitamin A has been discarded from the liver, whereas in human beings the liver is never completely free of vitamin A even if clinical signs of vitamin A deficiency appear, such as hemeralopia as seen in liver diseases (110, 111 and others).

The value of vitamin A therapy in those cases is apparent from the histologic picture (112). Large doses have to be given in order first to saturate the pathologic sites which have an increased avidity for vitamin A and then to enforce storage in the normal sites from which vitamin A is more readily available for use. Actually, giving large doses to carbon tetrachloride intoxicated rats (40), or human beings with liver damage (38) resulted in storage of vitamin A in normal sites.

*4 Fish Liver* In fish livers (113) fluorescence is imparted by medium sized fat droplets which fill the cytoplasm of the cells, the Kupffer cells being not fluorescent. In salt water fish, the green fluorescence is of extreme strength and the liver structure can be made out only after the fluorescence has partly faded. In fat stains of livers from fresh water fish an identical picture was found. However only a few of the fat droplets imparted the green, quickly fading fluorescence, whereas the majority show a yellow brown, slowly fading fluorescence. This is due to vitamin $A_2$, a factor with an absorption spectrum

at 352 m$\mu$, demonstrated by physico-chemical analysis in livers of fresh water fish in addition to small amounts of vitamin $A_1$ (114, 115, 116). Oils extracted from fresh water fish showed the yellow-brown fluorescence. If fed to vitamin A deficient rats, the yellow-brown fluorescence appears in the liver distributed in a manner similar to that of the usual green fluorescence and, as described (118 116, 117), growth of the rats continues. The different histologic distribution of vitamin A in the livers of fish supports the idea that vitamin A plays a special rôle in the metabolism of fish (33).

ADRENAL GLAND. In the cortex of the adrenal of the rat large amounts of vitamin A are present in small lipoid droplets in the epithelial cells of the fascicular layer. The glomerular layer, despite identical fat distribution, is free of vitamin A, even in hypervitaminosis in which the fluorescence extends almost to the medulla. In rats with low vitamin A reserves, the outer zone of the fascicular layer retains it (38).

In the human (26), vitamin A fluorescence is found in the fascicular layer of the cortex and to a minor degree in the glomerular layer, also in the central cortex close to the medulla. The fluorescence is less vivid and fades more slowly than that of the liver. Double refraction and vitamin A fluorescence were often concomitant and at times in identical distribution. The reticular layer reveals brown-red fluorescent particles (lipofuscin) which are also seen in the fascicular layer after fading of the vitamin A fluorescence. The variations of the vitamin A fluorescence are not necessarily parellel to that of the lipid content. Only small amounts or traces are observed in malnutrition and diseases of the liver, also in infectious diseases, here probably related to the depletion of the lipids (119, 120). The extensive changes of the human adrenal in early postnatal life (121, 122) influence the vitamin A distribution. During the entire first year only small amounts are found and then the storage gradually approaches that in the adult.

The adrenals are a storage place for various lipoids and one of the substances in the lipid mixture gives vitamin A fluorescence. Its slow fading may be caused by the presence of carotenoids which are responsible for the yellow color of the organ.

TESTICLES. In human testicles (26) after puberty, different fluorescent details appear which partly reveal a green, fading fluorescence suggestive of vitamin A brown pigment in the cells of Leydig, and lipoid droplets and lipofuscin in the Sertoli and spermatogenic cells. The fluorescence of the Leydig cells is strong but slowly fading. The vitamin A fluorescence seems related to the activity of the testicle, appearing with maturity and disappearing with involution. In some animals the interstitial cells of Leydig show strong vitamin A fluorescence, whereas in others it is missing.

OVARY. In the rat ovary (38) a dull but slowly fading vitamin A fluorescence is seen in the interstitial cell cords and a bright and rapidly fading one in the so-called corpus luteum. In vitamin A deficient rats no vitamin A is seen in the ovary, upon feeding, it reappears.

In the human ovary the vitamin A fluorescence changes during development

and with the menstrual cycle, as studied on material obtained at autopsy (26) and from surgical operations upon women whose menstrual history could be ascertained (123) Three types of characteristic fluorescence are encountered 1, a very dull, very slowly fading, diffuse, green fluorescence in the cytoplasm of the granulosa cells of the corpus luteum, probably due to carotene (carotene is found chemically in the ovary (124) and causes the yellow color of the corpus luteum), 2, a bright quickly fading fluorescence seen particularly in fine fat droplets in granulosa cells, and 3, a little duller, more slowly fading one in fat droplets in theca cells

Vitamin A fluorescence appears around the third month of postnatal life, in fine fat droplets in the stroma, and in granulosa and theca cells of the Graafian and atretic follicles In the reproductive stage the granulosa cells of the maturing follicle reveal fine fluorescent droplets They increase in size and number after ovulation as the corpus luteum forms The appearance of the granulosa cells in the mature corpus luteum suggests the possibility that the fluorescent droplets are secreted into the capillaries With involution the picture of secretion changes into that of retention The shrinking granulosa layer contains large, strongly fluorescent droplets and fine fluorescent droplets appear in the surrounding tissue Finally, the vitamin A fluorescence disappears The corpus luteum of pregnancy shows only carotene and no vitamin A fluorescence The corpora atretica, which are rich in estrogen and progesterone (125), show strong vitamin A fluorescence of the double refractile lipids in the theca cells In the course of the atresia it gradually disappears, while a red brown ultra violet stable fluorescence persists The vitamin A fluorescence of the corpora atretica depends on their size and age and the menstrual cycle The smaller and older the corpus atreticum, the lower the fluorescence After the climacterium no vitamin A fluorescence is seen in the ovary

The significance of the morphologic lipid distribution in the ovary and its relation to endocrine function is not established (126, 127, 128) The vitamin A fluorescence, however, seems to be a morphologic sign of endocrine activity Whether it indicates hormone storage or formation is not clear As long as the relation of vitamin A to sex hormone activity and even to reproduction is as unclear as at present (literature, see 26, 123), the presence of vitamin A fluorescence at the morphologic sites of sex hormone activity is best explained by solubility of sex hormones and vitamin A in the same lipid carriers.

Uterus The endometrial lipids, present in certain stages (129, 130), occasionally reveal quickly fading vitamin A fluorescence without parallelism to that in the ovary (123)

Breast The non lactating mammary gland is free of vitamin A fluorescence The fat droplets in the epithelial cells of the lactating gland reveal high amounts of it (14 26) in agreement with the high vitamin A content of the colostrum (131)

Fat tissue. The fat cells in adipose tissue or scattered in the stroma of various organs impart a dim but slowly fading vitamin A fluorescence, in agreement with their function as vitamin A depots The lipidic pigments retard the fading

and dim the fluorescence The different depots of the same individual vary in their fluorescence and show some relation to the nutritional condition (26)

Eye The presence of vitamin A in the eye is established (see 132) The vitamin A fluorescence, as reported by Querner (8) and Evans and Singer (133), changes depending on the function as described by Jancsó and Jancsó (13), Greenberg and Popper (134), and most carefully by Schairer and Patzelt (16) The pigment coat shows fine fat droplets arranged on the periphery of the epithelial cells They reveal yellow, ultra-violet, stable fluorescence which may be covered by a green, fading vitamin A fluorescence In the rod and cone layer, some diffuse vitamin A fluorescence is found in addition to a brown-red ultraviolet stable fluorescence which, according to Schairer and Patzelt (21), is due to visual purple Vitamin A fluorescence is also imparted by connective tissue cells in the ciliary processes

The nutritional status determines the vitamin A fluorescence of the ciliary processes just as it does that of other organs, but hardly influences that of the pigment layer In the latter it is not increased in hypervitaminosis and not apparently decreased in hypovitaminosis Even in far advanced vitamin A deficiency, long after the other organs have lost their vitamin A fluorescence, it is still observed in the retina and can be found even in the ulcerated eyes from extreme vitamin A deficiency The state of light adaptation, however, which does not change the vitamin A in the ciliary processes, influences the fluorescence of the pigment layer and rod and cone layer In dark-adapted animals no vitamin A fluorescence is seen, but the visual purple fluorescence of the rod and cone layer is strong Upon light adaptation the latter disappears, whereas the vitamin A fluorescence is strong The appearance of vitamin A upon exposure to light is rapid and takes place also in dead animals, its disappearance in the dark is slow The fluorescence microscopic examination corroborates the hypothesis of Wald (135), that light changes the visual purple over a transitional stage of visual yellow and the carotenoid retinine to vitamin A and that during dark adaptation the visual purple is slowly rebuilt from vitamin A

Intestine The gastro-intestinal tract of the rat reveals vitamin A in its fat cells Only after feeding of more than 900 units of vitamin does it appear in the wall of the duodenum and small intestine as a sign of its absorption It is first seen in epithelial cells and in histiocytes and lacteals of the lamina propria of the villi, later on in larger lacteals up to the subserous layer (38) This picture of lymphatic absorption agrees with experiences in the human (136) Recent experiments (137) showed that vitamin A absorption after administration of oily concentrates starts in the upper duodenum, has its peak in the upper jejunum and subsides in the ileum That does parallel the fat absorption Whereas fat is found in the lumen throughout the entire intestine, its vitamin A fluorescence disappears above the level of the intestine at which the vitamin A absorption ceases, even after feeding of more than 300,000 units The absorption subsides, therefore, either because vitamin A has been destroyed within the lumen of the intestine or because the entire vitamin A has been absorbed In the human intestine no vitamin A fluorescence is encountered (26)

KIDNEY In the kidney three types of distribution of vitamin A fluorescence occur in different animals In the rat (38) a bright, quickly fading fluorescence is imparted by the endothelial cells of the intertubular capillaries of the cortex It is marked in hypervitaminotic animals but also in advanced hypovitaminosis or in choline deficiency (62) when in both instances the liver is nearly depleted. Similarly the renal vitamin A concentration increases in depletion of the liver after dibenzanthracene administration (76)

In the normal dog kidney vitamin A fluorescence is imparted by medium sized isotropic fat droplets in the Henle loops

In the human kidney (26) vitamin A fluorescence is only encountered in renal damage The fluorescence is seen chiefly in the proximal convoluted tubules in fine droplets at the base of the epithelial cells In the vicinity, the stromal histiocytes are rich in fluorescence The vitamin A fluorescence is usually associated with marked fatty changes but not necessarily with deposition of doubly refractile lipids

The significance of the different forms of vitamin A fluorescence is unknown In the human its appearance seems to be associated with the development of the nephrotic syndrome Vitamin A appears in the urine together with other lipoids in renal diseases, but also in infections (see 138 139) Together with other lipids and with protein, it seems to be reabsorbed in the tubules and carried from there to the tubular capillaries sometimes being deposited in the interstitium (140) This explanation would agree with the recent theory on the histogenesis of lipoid nephrosis as a primary disease of the glomeruli with secondary tubular reabsorption of the lipoids and proteins (141, 142)

SKIN Although the epidermis shows very early manifestations of vitamin A deficiency, *no vitamin A fluorescence is encountered in the epithelial layers*, even after feeding of high doses of vitamin A Only the fat cells in the cutis and subcutis reveal small amounts (143)

OTHER ORGANS In the rat (11, 38), after feeding high doses vitamin A may appear in the endothelial cells and connective tissue cells of lung serous membrane, meninges and in other places In the human, fluorescent fat droplets may be seen in the anterior lobe of the pituitary gland (8, 14)

TUMORS Vitamin A fluorescence is seen in tumors originating from a mother tissue with vitamin A fluorescence its distribution resembling that of the mother tissues (144) It may be found in hepato-cellular carcinoma in adrenal cortical adenoma resembling the glomerular layer, in lipoma and in testicular dysgerminoma. Among the ovarian tumors it is chiefly present in those with endocrine activity It occurs in the masculinizing arrhenoblastoma and in the feminizing group granulosa cell tumor (in a distribution similar to that of the normal granulosa), theca cell tumor (resembling theca cells) and xantho-fibroma (simulating ovarian stroma with vitamin A fluorescence), while the ordinary fibroma of the ovary is without fluorescence It was also observed in ovarian dysgerminoma (in a distribution similar to that of the testicle) The picture of a tubular adenoma of the breast resembled the lactating breast Except for a few examples (*e g*, plant cells), the other tumors were free of vitamin A fluorescence,

even if they showed marked fatty degeneration. The presence and distribution of vitamin A fluorescence may help in determing the origin of a tumor. Thus the hypernephroid carcinoma of the kidney often reveals vitamin A fluorescence similar to that seen in the adrenal. This supports the original hypothesis of Grawitz, that those tumors originate from the adrenal (literature, see Ewing 145). Other renal tumors like carcinoma of the pelvis or benign tubular adenoma have no vitamin A fluorescence despite the presence of anisotropic lipids in the latter. That only a few well defined groups of tumors yield vitamin A fluorescence indicates that the local presence of vitamin A does not inhibit tumor growth, neither is it necessary for tumor formation. This is important in view of the repeatedly (see 144, 146, 147) discussed relationship between vitamin A and tumor formation.

Relation of vitamin A fluorescence to fat. The vitamin A fluorescence is, with few exeptions, bound to lipids demonstrable by sensitive stains. Vitamin A (or the other substances yielding the fluorescence) can be considered as exhibiting a fluorescent vital stain characterizing certain lipids of the body. However, since only some lipids reveal vitamin A fluorescence, the presence of vitamin A is not due to a non-specific solubility of vitamin A in the available fats but to a specific affinity of certain lipids for vitamin A (14, 148).

There is an apparent influence of vitamin A upon the fat metabolism in rats put on a vitamin A deficient diet. In agreement with chemical analyses (149), the morphological demonstrable fat disappears from the liver shortly after vitamin A vanishes, after feeding a fatty diet containing vitamin A, fat reappears one or two hours before vitamin A becomes visible (38). Various investigations (78, 76) indicate that vitamin A may occur also in a protein bound form, this is not excluded by the fluorescence microscopic picture.

The relation between the vitamin A depots in the body. In rats on vitamin A deficient diet the fluorescence disappears from different sites in the following order: interstitium of the various organs, adrenal, liver, and kidney, while the retina does not give it up at all. In repletion by oral route it reappears first in the intestine, then in the liver and almost simultaneously in the adrenal, finally in the kidney and the rest of the organs (38).

Upon carotene feeding, sometimes the vitamin A fluorescence appears first in the lamina propria of the villi of the small intestine but usually first in the Kupffer cells and shortly afterwards in fine droplets in the epithelial cells of the liver and in the adrenal (38). The question as to where carotene is split to vitamin A is therefore not yet satisfactorily answered. Most of the evidence points to the Kupffer cells of the liver (see also 100). However, sometimes cells of the intestinal villi or epithelial liver cells seem to split it.

Review of the vitamin A distribution in tissues. So far, not much can be concluded from the histological picture as to the rôle of vitamin A. Where the first morphologic signs of vitamin deficiency appear, namely, the epithelium (55), vitamin A fluorescence is not demonstrable. Actual presence of vitamin A is required for its rôle in vision, it participates in a known chemical process in the retina from which it does not disappear even in extreme deficiency. The

different localizations of vitamin A may be due to the following factors 1, participation in a chemical process (retina), 2, distribution and possible destruction (Kupffer cells), 3, presence at the site of sterone hormone formation (adrenal, testicle and ovary), probably due to solubility in the lipid carriers of the hormones, 4, storage (fat cells and cells of the interstitial tissue in the rat), 5, absorption (intestine), 6, secretion (lactating breast), 7, pathologic permeability (kidney), 8, imitation of the mother tissue (tumors) Its presence in the epithelial cells of the liver is due to several of the above factors

## CONCLUSION

The microscopic visualization of vitamin A by fluorescence permits the study of its distribution in organs In various physiologic and pathologic conditions the distribution undergoes characteristic changes, not indicated by chemical analysis or bio-assay Conclusions as to the rôle of vitamin A in the body can be made which are not arrived at by other methods The histologic method, which permits only a rough but quick estimation as to the quantity of vitamin A present, does not substitute for the chemical analysis but supplements it The fluorescence microscopic demonstration of vitamin A, however, not only aids in the investigation of the vitamin A metabolism but also presents a new histologic method for the differentiation of lipids independent of the biologic significance of the fluorescence

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# THE HISTOPATHOLOGY OF RADIATION LESIONS[1]

SHIELDS WARREN

*Laboratories of Pathology of the Harvard Cancer Commission and the New England Deaconess Hospital and the Department of Pathology Harvard Medical School*

The histopathology of lesions induced by radiation is the resultant of several diverse changes Some changes are cellular, as necrosis of cells irradiated alteration of cellular function, or chromosomal changes induced in cells which lead to death or alteration of function of cells subsequently developed from them Some are intercellular (probably in part mediated through injury to the cells maintaining such substances as collagen, osteoid, or elastica), varying from slight alteration in texture to necrosis Some are vascular Since endothelial cells and other components of vascular walls are fairly sensitive to radiation, vascular changes ranging from thrombosis to endothelial swelling as early manifestations and from telangiectasia to complete or partial occlusion as late alterations may be induced These altered vessels often inconspicuous in themselves, may bring about profound changes in those tissues dependent on such vessels for their blood supply

The character of the pathologic alterations produced in any tissue depends on its cellular pattern and the degree and extent of exposure to radiation Radiation lesions vary markedly according to whether they are recent or long standing and according to the tissue in which they occur All, however, have certain features in common and it will be the function of this review to consider these particularly

The types of radiation producing the lesions which will be discussed here are those used therapeutically electro-magnetic radiation of a wave length ranging from 10 to 0 05 Ångström units, corpuscular radiation as the alpha and beta radiation from radium or certain of the temporarily radioactive isotopes, and neutrons So far as one can determine, the fundamental biologic effects of these different agents are the same and are dependent on the degree of ionization produced by them in the tissues and the localization of the ionization within the cells

A fascinating recent development in the field of therapeutic radiation has been the utilization of temporarily radioactive isotopes (1, 2, 3, 4) The peculiarities of certain of the body's cells in utilizing one or another chemical substance can serve to concentrate radiation effect in those particular regions if the proper isotope is selected Thus Marshak (5) has shown that radioactive phosphorus is taken up by the nuclei of rapidly proliferating cells ten times as rapidly as it is by the nuclei of resting cells and by utilizing radioactive phosphorus one therefore can get a consolidation of radiation effect on those tissues

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whose cells are undergoing rapid proliferation Striking therapeutic results have been obtained in leukemia and polycythemia Similarly radio-active strontium is segregated by osteoblasts and laid down to a considerable degree in bone, thus concentrating radiation in regions of osteoblastic activity Unfortunately, in the case of tumors of the thyroid, the neoplastic cells rarely show the avidity for iodine that normal thyroid cells do and hence the hoped-for therapeutic effect from the use of radioactive iodine in thyroid tumors could not be obtained

It must be remembered that so far as immediate radiation effect alone is concerned, tremendous doses are required to kill all of a given group of cells, even though slight doses will kill some Radiation does not affect equally all cells within the field, even at an equal depth beneath the skin and an equal distance from the source, because of the discontinuous character of the energy applied As an example of this character, we may quote from Crowther, "Suppose that it were possible to pass, through the air of an ionization chamber, X-radiation at an intensity of 1 r per second continuously, day and night, for 500 years, we should still leave about one third of the molecules unirradiated" (6)

In any consideration of the dosage of radiation, it is important to know the physical factors of that radiation The effect is proportionate to the amount that is absorbed, rather than the amount as measured by the ionization chamber The "harder" the radiation (the shorter the wave length), the greater will be the penetration of the tissues and the less will be the damage to the skin by a given dose The "hardness" increases with voltage and with filtration "Cross-firing" through different skin portals toward a tumor will sometimes permit giving a greater depth dose to it than any one area of the skin receives The effect is also influenced greatly by the size of the field This is due in part to the fact that the "scatter", or the number of electrons separated from the atoms irradiated and giving secondary radiation, increases for a given dose of radiation with the increase in mass exposed Thus, because of this secondary radiation, the total amount of radiation per given dose delivered will be much greater per cubic centimeter of tissue if the field of radiation is 10 x 10 cm, than it is if it is 3 x 5 cm Irradiation of the entire body is very potent in man, a dose of about 500 r kills The rate of delivery of radiation is also important, for example a dose of 3000 r delivered in two hours is far more destructive than the same total dose fractionally delivered at daily intervals for 10 days One-tenth r per day is the accepted tolerance dose for man, but may be a trifle high (7)

Not too successful attempts have been made to establish biologically equivalent doses of radiation However, one can estimate to a limited degree the amount of beta radiation, of neutrons, of x-rays required to obtain similar degrees of injurious effect on tissues

Alpha particles, although virtually devoid of penetrating power, are about one hundred times as effective in injuring cells as beta rays Both are less penetrating and more destructive than gamma rays, which are equivalent to the hard roentgen-rays produced by supervoltage apparatus Accepting skin erythema as an index, well-filtered radiation at 1,000 KV requires ±1200 r,

at 200 KV, 700 r, and unfiltered radiation at 100 KV 320 r Neutrons are apparently from one to fifteen times as potent biologically as physically equivalent amounts of roentgen rays

The r, the unit of x ray radiation, is merely a measure of the ionization of air by roentgen rays under standard conditions and a dose expressed in r alone gives little information as to the biological effects that may be expected

A word should also be said with regard to the measurement of radiation given off by temporarily radioactive isotopes Their amount of radioactivity is commonly expressed in millicurie equivalents, that is, the effect that they produce on a standard ionization chamber as compared with radiation from 1 mgm of radium This is a physical measurement and does not imply any direct biological comparability of effects with a similar dose of gamma radon radiation measured in millicurie hours

In spite of the different types of radiation, the different wave lengths and the different intensities of radiation experienced, the pattern of response of the tissues, once the threshold of reaction of their components has been reached, varies quantitatively rather than qualitatively (8)

The assumption that small doses of x ray or radium radiation are stimulating (the Arndt-Schulze "law") is invalid The slight evidences of proliferative activity offered as evidence by the proponents of this hypothesis are in fact only reparative responses to the injury that has been done On examination of a wide range of tissues irradiated to varying degrees, I have found no evidence of a stimulating effect

Different tissues do not show unique changes Certain combinations of changes are characteristic (9) as will be pointed out later Thus, though radiation tends to produce abnormal mitoses, vascular injury, and hyalinization of collagen, these may all be produced by other substances, as lipoid soluble anesthetics, in the case of multiple mitoses, the heat of cauterization, in the case of hyalinization of collagen, and a wide variety of physical and chemical agents in the case of vascular damage The early responses to radiation injury seem to be in part concerned with altered fluid balance edema of the tissues and swelling and vacuolization (10) of the cells are prominent features

The histopathologist can say with a fair degree of accuracy that a given tissue effect is produced by radiant energy, but he cannot so state with infallibility

The combination of two or more of the non-specific characteristics of radiation is strongly presumptive evidence that the injury is in fact the result of radiation Thus the combination of giant and irregular nuclei, hyaline connective tissue, and thick walled hyalinized blood vessels would be difficult to explain on any other basis than that of a late response to irradiation

It was noted early that various types of cells and various tissues show injury after widely different doses of radiation. Viruses may be unchanged by radiation of several hundred thousand r Some fibroblasts in vitro may survive 100,000 r, whereas frog sperm may be profoundly altered by as little as 25 r This variation of response of different cells and tissues is of great importance clinically It is this variation in response which permits radiation to be used

as a therapeutic agent producing injury selective, at least to a degree, for certain abnormal cells or tissues while sparing others.

In general, one classifies tissues or tumors as radiosensitive if there is gross change with radiation of less than 2500 r given under ordinary therapeutic conditions. Such radiation does not usually injure most tissues permanently. A tissue is considered radioresponsive if such alteration occurs with a range between 2500 and 5000 r and radioresistant if over 5000 r is required for a response. At or above this dose some permanent injury is done to a number of normal tissues (9).

That variations in sensitivity to radiation are not necessarily dependent on specificity of protoplasm is shown by the extraordinary variations in effect on Drosophila at different stages of the life cycle. The ova have a median lethal dose of 155 r and the late pupae have a median lethal dose of 95,000 r. Fortunately for tumor therapy, cells in mitosis are much more sensitive than resting cells. Other physical agents, of course, vary in the extent of injury they cause to different stages of development of the same organisms, but not to so great a degree. Thus eggs of *Ankylostoma caninum* are readily killed by freezing with dry ice, whereas the larvae live frozen for weeks (11).

The assumption has been made (the law of Bergonié and Tribondeau) (12) that more primitive cells are more sensitive to radiation. For example, the lymphocyte is the most easily injured of somatic cells and the neuron is one of the most resistant cells of the body as might be expected from its high degree of organization and its low metabolic activity. While this assumption holds to some degree in the animal organism, it is not a consistent rule. Thus the relatively simple organism *Chaos chaos*, or giant ameba, is extraordinarily resistant to radiation while the specialized ciliated epithelium of the mammalian bronchus is highly sensitive.

Much of our knowledge of variations in response of different cells, tissues, and tumors is empirical. Several valuable reviews of radiation effects in general are available (9, 13, 14, 15, 16, 17, 18) as well as of effects on tumors (19).

Injury, whether to tumor tissue or normal tissue, due to therapeutic irradiation, unlike that due to other physical agents, is not immediately apparent on inspection at the site of the injury or elsewhere. Several days may elapse between the time of irradiation and the first visible changes in the skin. With ordinary therapeutic doses, this latent period in all probability is only apparent, as intracellular changes have been shown to develop almost immediately following the onset of irradiation. The height of reaction may not be reached for several weeks and if the dose has been heavy, new evidence of injury may develop even after months or years. Much of our present interpretation of the so-called latent period rests on experiments performed in the early days of radiation and carried out with variable physical factors owing to both mechanical inadequacy of the source of radiation and inadequate physical means of measuring the amount of radiation given. It might well be profitable to reinvestigate some of our knowledge of the latent period.

In some cells and particularly in the germ cells, the latency in appearance

of injury is real Only a portion of a single chromosome may be damaged and no effect may appear until a number of mitoses have taken place, finally resulting in the development of a cell in which the original masked chromosomal injury will become apparent through marked alteration in form, function, or even in cell death (20)

One common assumption has been made by both laymen and physicians that in the course of treatment of a given lesion by radiation any permanent damage to normal tissue or the production of an x ray "burn" represents evidence of incompetence This is not true Radiation cannot be given effectively to any tissue without at least some effect on adjacent tissue and the character of *that effect hinges not only on the skill of the radiologist but on the dosage of* radiation which it was necessary to deliver to accomplish the therapeutic end desired As more and more patients are cured of tumor by radiation, we will inevitably see undesired late sequelae of radiation, varying from atrophic skin and disfiguring telangiectasis, to actual extensive necrosis

The sensitivity of cells varies not only between types of cells themselves but even in the same cell with various stages in its life cycle Thus the nuclear chromatin in general is affected more than is the cytoplasm, and due to its relative importance, nuclear injury has profound effects on the cell as a whole The nucleus in mitosis is particularly sensitive In the human and many animals, the early prophase is apparently the stage in which the cell is most susceptible to radiation injury With a fairly intense source of radiation, there will be decrease in mitotic activity within one half hour of the onset of radiation and there will be practically complete cessation of mitotic activity up to twelve hours with recovery under way by eighteen hours provided the cell survives (21) While injury to nuclear structures may be done at all stages of mitosis, by and large mitosis once initiated tends to continue through to completion Evidence of radiation injury may be apparent in these continuing mitoses, however

The types of visible change produced are 1, inhibition of mitoses, 2, breakage of chromosomes, 3, lagging of chromosomes, 4, complete or partial adhesion of chromosomal pairs 5, asymmetry of mitosis There is a marked tendency for multipolar mitoses to develop following radiation (22) Three stages of mitotic injury were noted by Politzer (23) the immediate stage with inhibition of mitoses pseudo-amitosis and pyknosis, the intermediate stage, during which mitotic activity is practically absent, and the later stage during which abnormal or multiple nuclei are formed as a result of a division of nuclei whose chromosomes have been injured This period of abnormal mitosis is most marked from 24 to 96 hours after irradiation in case of some tumor tissues. The occurrence of *multipolar mitoses is, of course, accompanied by and perhaps dependent upon* the occurrence of multiple centrioles and as many as 50 per cent to 80 per cent of interkinetic cells may present multiple centrioles following radiation (22) It is important to remember that the visible injurious effects on the nucleus and on mitosis are not pathognomonic of radiation and may be produced by heat, ether and other means

The effects on the cytoplasm and its constituents are less well understood than

those on the nucleus, in part because it is difficult to tell what cytoplasmic alterations are primarily due to nuclear injury and what are actually direct effects on the cytoplasm itself

The first cytoplasmic change noted in tissue culture is the cessation of motility and the retraction of pseudopods with a resultant rounding up of the cells

Perhaps the most consistent feature noted in the cytoplasm is vacuolization (10) This occurs in a high proportion of cells damaged by radiation and is most apparent in those going on to necrosis This vacuolization suggests an altered permeability in the cell membrane The vacuolization is due at least in part to inhibition of fluid as the cells increase materially in size, roughly paralleling the degree of the vacuolization Other constituents of the cytoplasm are not particularly involved The mitochondria will be injured by heavy radiation but this injury is not characteristic The Golgi apparatus is sometimes fragmented and appears swollen with a sublethal dose of radiation Recovery usually occurs

In organized tissue preparations, loss of cell function is early apparent Thus the cilia of respiratory epithelium cease to function very promptly after the onset of radiation The power of phagocytosis is lost within a few hours and the secretion of mucus in the intestinal tract is increased very strikingly within some days The secretion of acid by the gastric mucosa may be brought about a few days after radiation and will persist for some two to four months Dryness of the mouth due to deficient salivation is a frequent accompaniment of therapeutic radiation about the mouth

Among the interesting effects produced by radiation on intracellular tissues are the changes in bone (24) The characteristic feature is the maintenance of integrity of structure with eburnation and devitalization without leukocytic reaction The osteoblasts disappear, the osteocytes are absent and the lacunae which they occupied are apparent as empty spaces Frequently the canaliculi are enlarged and very irregular but reduced in numbers Abnormal spaces may appear about the lamellae As a rule the density is markedly increased and an almost ivory-like texture is apparent This is emphasized in the x-ray, showing as dense zones This maintenance of structure in spite of heavy radiation is due in part to the slowness of metabolic processes in bone and in part to the existence of its amorphous substrate

Osteoclasts are apparently slightly more sensitive than are osteoblasts and at least a portion of this eburnation may result from a reduction in the usual resorption of the bone More rarely injury will be apparent as rarefaction as a result of excessive resorption and in these instances it is due to halisteresis rather than to osteoclastic over-activity The changes in the osteocytes themselves are not striking, pyknosis, vacuolization and disappearance following more or less in sequence A striking feature is the absence of any zone of demarcation between the normal and irradiated bone, one shading off into the other There is no sequestration in the ordinary sense Usually when sequestration occurs, it is because of the advent of secondary infection Such infection may develop in irradiated bone, and when it appears the picture resembles

chronic osteomyelitis of long standing. Irradiated bone is more susceptible to infection than is normal bone because of its poor circulation, low cellularity and necrosis. Necrotic bone acts as an infected foreign body.

The cells of the epiphyseal line are much more easily injured than are osteocytes. The columnar pattern becomes disorganized and some cells are damaged and disappear (25). If the dose of radiation has been slight, regeneration occurs, but if heavy, growth at the epiphysis will be lessened or cease. However, a moderate dose of radiation will not hamper healing of a fracture.

In considering the result of irradiation of cells their relative sensitivity must be kept in mind. If somatic cells were to be arranged in order of decreasing sensitivity to radiation, there would be first lymphocytes, then granulocytes, then epithelium, smooth muscle, fibroblasts and their derivatives, and finally neurons. The sensitivity of the germ cells is probably of the same order as that of the lymphocytes. The immature ova are apparently more resistant than are the matured ones, since a dose of 1200 r at 200 KV potential, for example, administered to the ovaries of a woman in the childbearing period will induce amenorrhea and sterility, usually followed in a number of months by a resumption of the menstrual cycle and fertility.

The effect of radiation on germinal cells must be considered as somewhat different from that on somatic cells. While x-ray injuries to normal tissues were noted within a few months after the discovery of roentgen radiation, the sensitivity of the ovary and testis was not noted until eight years had passed by. Bergonié and Tribondeau (12) pointed out that the more primitive cells, among which are the germinal cells, were more susceptible to radiation than better differentiated cells. In recent years, chiefly through the work of Muller (20), the effect of radiation on germ plasm received added emphasis through its application to genetic studies. The initial technique was simple. Male Drosophila were irradiated and mated with normal virgin females. The offspring were then examined for abnormalities. In general the normal mutation rate is practically doubled in Drosophila by a dose of 30 to 40 r applied to the germ cells lying in the gonads. Viable mutations occurred only one tenth as frequently as lethal mutations. Further evidence has been brought out by the mutation of chromosomal aberrations (26).

Henshaw (7) concludes that x-ray-induced mutations are a result of local rather than general exposure, that the results of previous exposures are additive, that the duration and frequency of exposures do not influence the reaction, that recovery does not occur, and that general idiosyncrasy is important.

It may be fair to assume that irradiation of human gonads would give the same proportion of lethal mutations to viable mutations as in Drosophila, namely, ten to one. This might account for the relatively low incidence of abnormal embryos in the human. The experimental data have been reviewed by Murphy (27) and human data have been presented by Jost (28) and by Hickey and Hall (29). In Jost's series of 63 pregnancies following pelvic irradiation of 141 women, there were 14 abortions and 41 live children, only one of these was abnormal, showing underdevelopment with strabismus and myopia. Hickey

and Hall (29) obtained information from 377 radiologists with regard to fertility and abnormalities of offspring Thirty-seven per cent of the couples had no children, and of the 412 children 4 per cent showed abnormalities

There is no question but that moderate to heavy radiation of the embryo is deleterious If the irradiated embryo survives, the abnormalities that subsequently develop may be quite characteristic The eyes are frequently involved, the epiphyseal lines may show evidence of injury with striking alteration in the growth of the bones involved (usually those of the extremities) Goldstein and Murphy (30) reported on 75 children who had been irradiated in utero Thirty-eight showed defects or serious ill health, 20 of the 75 showed central nervous system changes and 16 per cent of the group were microcephalic

This distinction between somatic cells and germ cells has an important relationship to the whole problem of radiation effect, because the protection planned to avoid somatic tissue injury may not be adequate for protection of the germ plasm The ordinarily accepted tolerance level for protection is 0 1 r per day In establishing any level for the tolerance dose, it is important to remember that other criteria exist than the obvious ones often adopted, the development of dermatitis or sterility The lymphocytes have long been established as the most sensitive of the somatic cells As has been noted by Russ (31), Henshaw (7), Warren (32) and others, the lymphocyte count in the peripheral blood is probably the most sensitive index that we have of somatic radiation injury This is manifest by a lowering of the lymphocyte count followed by recovery in 12 to 20 days, frequently still later followed by an over-compensation with a lymphocytosis of fairly long duration

In most somatic cells, 3 different stages of injury may be recognized as of increasing severity alteration of function, alteration of structure, and necrosis Not all stages occur in all cells irradiated, and rarely the descendants of surviving cells may become neoplastic

The rôle of infection in determining the response to radiation either in non-neoplastic or neoplastic tissues is important, but little understood Clinical experience has demonstrated that infected tumors are more resistant to radiation than are the same tumors free from infection The mechanism involved is unknown That irradiation of slight intensity is of value in the treatment of certain infections has been proved, particularly in the case of acute parotitis Here the hyperemia induced is probably of importance

Tissues damaged by radiation are susceptible to infection Infection, if it occurs, accentuates particularly the late deleterious changes In interpreting the course of infection in tissues that have been irradiated, two factors must be kept in mind first, the impairment of the vascular supply greatly hampers the normal defense mechanisms of the body, second, the dense hyalinized intercellular substance of irradiated tissue supported by cells already injured, when exposed to infection is readily killed and acts as a sequestrum keeping alive the infection An excellent example of this type of change may be seen when the presence of a carcinoma of the gum has made necessary heavy radiation of the region of the jaw Usually, in order to eradicate the tumor, it is necessary to

use a sufficiently great dosage to cause marked injury to the surrounding normal tissues Through the ulceration opened up by the necrosis of the tumor, bacteria from the mouth may gain access to radiation-damaged bone and to hyalinized collagen of the fascia. Since in irradiated bone there is no clear cut transition from healthy bone to that injured but rather a gradual transition from healthy bone through that slightly damaged to dead, there is usually no sequestration However, as the infection progresses through tissues with impaired blood supply and encounters already injured cells, it is not walled off and gradual disintegration of the more severely damaged bone occurs This is usually discharged in fragments, followed by further necrosis induced by progress of the infection until ultimately the chronic osteomyelitis may extend well beyond the fields of radiation Similarly if the ulceration and infection extend to the fascial planes of the cheek, there will be progress of low grade infection along the relatively inert collagen of the fascia, many of whose supporting fibrocytes have been either killed or heavily damaged by the radiation The fascia becomes necrotic, shreds, and is discharged in sequestrated fragments Such a process of necrosis and sequestration may continue for several years

Sometimes the effects of infection will be combined with those of trauma in producing lesions of clinical importance in irradiated tissue As an example of this, we may consider the radiation ulcers of the intestinal tract (33) In the course of the irradiation of tumors of the abdomen and particularly those of the pelvis, it is inevitable that more or less exposure of the intestinal tract occurs, resulting in varying degrees of radiation injury In observation of the radiation ulcers one is impressed by the fact that they are much more localized than is the field of radiation and the resultant abnormality of the intestinal wall, and their localization is such that it is difficult to interpret them as occurring as a result of radiation alone If we follow through the sequence of events in the lesions noted clinically and in those produced experimentally, it becomes apparent that ulceration occurs because of trauma and infection impinging upon an altered mucous membrane and bowel wall, structurally and functionally damaged by radiation and imperfectly regenerated While it has been suggested that radiation ulcers might be due to obliteration of blood vessels as a result of the radiation, our observations do not confirm this

Another example of the aggravation of radiation injury by trauma with or without infection is demonstrated in the case of chronic radiation injury of the hand This lesion, exemplified well in many of the early x ray workers, has been long studied and is as well understood as is any radiation lesion Owing to the radiation changes of the epidermis, the cells are thin, abnormal, disarranged and hyperkeratotic The cutaneous glands are absent, so that their secretions which aid in keeping the normal epidermis soft and pliable are of no avail in protecting the thin, scaly, irradiated epidermis Moreover, as a result of the radiation injury, the rete pegs are largely smoothed out and the basement membrane is degenerated The epithelium rests almost without structural bonding on the hyalinized corium which is a poor substrate for even normal epithelium The impaired blood supply further contributes to the lowered resistance to

injury and inability to repair, so that traumatism of the skin readily results in ulceration Ulcers, once formed, tend to persist almost indefinitely owing to unfavorable conditions both for epidermization and combating of infection As a result of abortive efforts of repair, often continued for years, the epithelium at the margin of these radiation ulcers may become carcinomatous

Radiation injury of the skin has long been recognized, both because of the number of cases of radiation dermatitis or radiation carcinoma that developed in radiologists and in their patients and, second, because of the utility of the cutaneous erythema as a means of gauging radiation dosage Therefore, at the risk of repetition, the histologic sequences will be considered The gradual transition over a period of years from healthy, elastic, normal skin to dry, atrophic, smooth or focally hyperkeratotic skin may be followed with ease The first change noticeable, immediately after irradiation, is a cessation of mitotic activity in the epithelial cells, followed in a few days by edema of the subcutaneous tissue with basal dilatation and evidence of vascular damage ranging from none to complete thrombosis At this time the gross erythema and edema of the acute reaction are apparent The collagen fibers, at first merely separated by the edema, become swollen and tend to coalesce Later there is increased production of collagen with the formation of dense, almost glossy, hyaline intercellular substance The elastic fibers swell, fray, and, depending upon the severity of the irradiation, either become increased in amount or disappear With progress of time, the epithelium becomes thin and the rete pegs tend to disappear, leaving an almost straight boundary between the epithelium and corium, with loss of the basement membrane This change, if it involves the fingers, may result in loss of the individually characteristic fingerprints The skin appendages atrophy and disappear There is an increased production of melanin by the basal cells Distortion of the arrangement of the epithelial layers is followed by hyperkeratosis, sometimes focal in character, and the appearance of abnormal epithelial cells, sometimes with atypical mitoses Such of the more superficial vessels and capillaries as survive are telangiectatic The deeper vessels are usually partially, or some even completely, occluded, with some endothelial proliferation, by hyaline thickening of their walls The epithelium, as it proliferates focally, penetrates somewhat into the corium, and finally becomes actively invasive (34) Years are required for the evolution of a carcinoma of the skin secondary to radiation The observations of Wolbach (35) in this field are classical and deserve study by all those interested

Radiation injury of several tissues as well as the skin, particularly that produced by repeated even though small doses, may lead to the development of neoplasms The pattern of evolution followed is similar to that of many of the induced tumors injury necessitating long continued reparative activity, abnormal environment of the proliferating cells and probably mutations induced in those cells In the human I have seen epidermoid carcinoma, basal cell carcinoma, fibrosarcoma, osteogenic sarcoma, and myelogenous leukemia, which apparently developed on the basis of radiation injury Also ascribed to the effect of radiation have been carcinoma of the lung (as seen in the Schneeberg miners and some radium workers), lymphatic leukemia and other lesions

Since the greatest use of therapeutic radiation is in treating tumors, separate descriptions will be given of the radiation changes in examples of the radio sensitive, the radio-responsive, and the radio-resistant tumor

As an example of histopathologic changes induced by irradiation of a radiosensitive tumor we may select as a fairly typical example the response of a lymph node involved by lymphosarcoma Within a few hours after the period of radiation there will be swelling of the node, which gradually goes down and at the end of several days the node, assuming the response has been satisfactory will be barely palpable If we follow microscopically through the course of events that underlies this series of changes, we will see first a cessation of mitotic activity in the tumor cells with necrosis of cells, both those undergoing mitosis and cells in the resting stage This necrosis is followed by autolysis of most of the necrotic cells, although some phagocytosis by macrophages of cells and cell fragments occurs The increase in size noted grossly in the nodes during this period is the result of edema, probably the result of the autolytic process This results after a few days in the histologic picture of the node being that of the reticular net and sinusoids distended with edema fluid, some phagocytic macrophages, and almost a complete loss of the lymphoid elements However, a few persist and usually after some weeks or months the tumor process has reestablished itself and may be even larger than it was at the time of treatment Owing to the light dose capable of accomplishing this change, very little, if any, change will be noticed in the supporting tissues Usually the recurrent tumor (cure is very rare) is more resistant to irradiation than was the primary tumor

As an example of the effects in a radio-responsive tumor we may take epidermoid carcinoma, grade III, of the cervix uteri The effects of equivalent doses of radium and of x ray irradiation, both of which may be used in the therapy of this tumor, are essentially similar Very shortly after exposure mitoses cease, sometimes within an hour Those cells in which the mitotic process has been initiated tend to carry through to completion but many of the cells die first Since the radiation given therapeutically is well over the threshold dose, injury is done to the resting cells as well as to the dividing cells The nuclei gradually become hyperchromatic and enlarge chromatin tends to be clumped rather than dispersed, and the nucleoli become strikingly prominent Calcification of the nucleus has been reported The cytoplasm becomes vacuolated and the cells increase considerably in size After 18 to 24 hours mitosis is resumed to some degree but the proportion of abnormal mitoses with unequal spindles, lagging chromosomes, chromosomal attachments or multi polar spindles is appreciably higher than was previously the case As a result of the multi polar mitoses, tumor giant cells with bizarre and giant nuclei may be formed. Other of the abnormal mitoses lead to death of the cell Necrosis develops both as a direct result of irradiation and as a process secondary to vascular and stromal damage. Secondary infection may also play a part The cytoplasm of the surviving cells tends to become more and more keratinized and sometimes the only residual cells will be giant forms with prominent keratinized cell membranes, intercellular bridges and kerato-hyaline granules While these changes are taking place in the tumor cells, the stroma does not remain unaltered The

fibrocytes of the stroma at first show no change but later some die and others become large, stellate rather than elongate, often vacuolated and may have irregular giant nuclei The collagen tends to lose its fibrillar structure, the fibers swell and coalesce to form a mass of dense hyalinized material Here and there in the collagen, foci of necrosis develop with deposition of fibrin The elastic fibers swell at first and later partially disintegrate The usual radiation scar contains but little elastic tissue capable of function

Very early in the course of radiation there is swelling or even necrosis of endothelial cells of blood vessels Where necrosis occurs and the vessel is small, there will be an occluding thrombus, if large, there may be only a mural thrombus The smooth muscle cells of the media are swollen and necrosis of them may occur Several weeks after irradiation the late vascular changes will usually have developed There will have been either thrombosis and organization, or proliferation of the swollen endothelial cells to a sufficient degree to cause at least partial occlusion In the smaller superficial vessels and capillaries, the damage to the wall and back pressure from narrowed veins may bring about a marked degree of telangiectasis In the larger vessels, a marked narrowing or occlusion of the lumen results, due in part to endothelial thickening and proliferation and in part to thickening and hyalinization of the connective tissue and degeneration of the elastica and muscle cells of the media

The initial tissue destruction by the tumor, the death of the tumor, the dense hyaline character of the connective tissue and the impaired blood supply lead to ulceration of the cervix which presents quite a characteristic appearance Superficially there is a zone of relatively cell-free fibrillar necrosis rather reminiscent of that seen at the base of a chronic peptic ulcer This fades off over a distance of 20 to 50 micra, passing through a stage of coarsely fibrillar, partly hyalinzed connective tissue to a zone of marked hyalinization Here and there a few leukocytes may be scattered but in general their response is minimal More superficially there may be telangiectatic capillaries and venules and thick-walled or completely occluded arterioles As one progresses farther from the surface, there may be a few scattered tumor cells embedded in the hyaline tissue These sometimes cluster into small islands If the radiation has been sufficiently heavy, the tumor cells will not be present If a recurrence of the tumor develops, the tumor cells as a rule are smaller, less keratinized and much more closely packed than they were during the period of response to radiation

As an example of radio-resistant tumors, we may take an adenocarcinoma of the rectum Here as a rule very heavy radiation has to be given to produce any significant effect Most of the tumor cells will remain undamaged, some of them even undergoing mitoses in the face of a dose of radiation that will cause marked degenerative changes in the smooth muscle, connective tissue and blood vessels of the intestinal wall Sometimes increased mucous secretion by the tumor cells results Partial regression of the tumor may occur, chiefly through injury to its blood supply

It is important not to confuse radio-sensitivity of a tumor with radio-curability of that tumor Thus a lymphosarcoma is highly radio-sensitive but rarely

cured while epidermoid carcinoma grade I of the lip is resistant but often cured if the dose is sufficient Many of the tumors that respond well to radiation initially will recur, and usually in a form more resistant to radiation than were the cells of the original tumor Moreover, the tumor may have metastasized prior to treatment and radiation does not affect metastases that may have developed outside the irradiated field any more than surgical removal would affect metastatic growths outside the operative field.

While in general the radio-sensitivity of tumors tends to follow that of the type cell from which they are derived, none the less there are striking variations from this and our knowledge of the radiation responses of tumors of various types rests on clinical observation rather than on *a priori* reasoning based on assumed analogous behavior of the corresponding normal tissues when irradiated Ewing's tumor of bone is highly sensitive to radiation but osteogenic sarcoma of the bone is highly resistant Leiomyoma of the uterus usually responds well to radiation while leiomyoma of the intestinal tract does not respond so satisfactorily Neuroblastomas respond well to radiation, while gliomas and melanomas are very resistant

There are also variations in sensitivity determined by the stroma of the tumors A relatively acellular supporting tissue, as cartilage or bone gives a less favorable therapeutic reaction than does a cellular one, as new formed connective tissue or invaded muscle For example, basal cell carcinoma of the skin is easily cured by radiation when located on the cheek whereas one occurring on the forehead invading the frontal bone is extremely resistant. In general it can be said that increased amounts of intercellular substance in the supporting tissue of the tumor or secondary infection tend to increase resistance whereas a greater degeee of vascularity tends to decrease resistance

Although the elements of the histopathologic response to irradiation are fairly simple, so many variables may operate to emphasize one and mask another that the lesions may vary widely and be difficult to diagnose until analyzed With further study many of the responses of normal tissues and tumors should become more accurately predictable

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# THE BREAD PROBLEM IN WAR AND IN PEACE

SAMUEL LEPKOVSKY[1]

*Division of Poultry Husbandry College of Agriculture, University of California Berkeley*

Bread, in the Bible, is a term used to signify food Thus in Gen III, 19, the Lord says to Adam "In the sweat of thy brow shalt thou eat bread" It is also expressed in the prayer "Give us this day our daily bread" Bread (whole wheat) is practically a complete food upon which populations have often leaned heavily for their source of nutriment and, indeed, it has well earned its age-old title "the staff of life"

The separation of the ground whole wheat meal into the coarse bran and fine flour began very early and the fine flour was often considered the choicest part of the wheat. "The Bible speaks of fine flour or meal, as a portion of the meat offerings to the temple" (1) The Romans had four or five grades of flour, the finest, from which all the bran was removed, was eaten only by the rich (1)

The ancients realized that wheat flour suffered in nutritive value when the coarse bran was removed, and their wrestlers "ate only the coarse wheaten bread to preserve them in their strength of limbs" (1) Hippocrates, the father of medicine, who flourished over two thousand years ago, recommended the unbolted wheat meal bread "for its salutary effects on the bowel" (1) Experimental evidence of the superior nutritive value of whole wheat flour as compared to white flour was furnished by Magendie, who might well be called the father of modern nutrition He found that dogs fed on the dark coarse bread lived for a long time in good health, but declined and died in less than two months if the bread fed was made of high grade white wheat flour (2)

The "fine flour" referred to was not impoverished to the extent of modern white flours Stone-milling ground the wheat germ and much of the bran so finely that they could not be separated out by bolting The "fine flour" was not white but had a creamy color Modern roller milling operations do not grind the wheat germ but flatten it so that it can be bolted out of the fine flour The bran is flaked and more completely removed The small amount of carotene in the flour is destroyed by special bleaching processes The bleaching process gives "the artist's touch" to man's ignorant abuse of his "staff of life" The increased efficiency of modern milling methods in destroying the nutritive value of "the staff of life" is shown in table 1 (2)

This efficient destruction of the nutritive value of whole wheat flour was perfected and came into general use after 1880 (2) It might well have had a disastrous effect on the health of our population had there not been at the same time a general rise in the standard of living with the result that the consumption of bread dropped sharply while at the same time there was a great increase in the

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consumption of butter, eggs, milk, fruits and vegetables other than potatoes (2) Sylvester Graham (1) pointed out in 1837 that fine flour was not injurious provided only small amounts were eaten or much "animal flesh" was consumed at the same time Osborne and Mendel (3) recently confirmed this idea experimentally and showed it was due to the proteins and vitamins furnished by animal foods such as meat, milk and egg These foods are now known as "protective foods"

We depend on "protective foods" to supply white bread-eating populations with proteins of high quality, minerals and vitamins The "protective foods" include eggs, milk and milk products, meat, fish, fruits, vegetables and whole wheat The "protective foods" with the exception of whole wheat are more expensive than the vegetable foods, and malnutrition has often been found associated with poverty (4)

TABLE 1

*Composition of 100 grams*

| | STONE GROUND "WHITE FLOUR" | ROLLER MILL BLEACHED WHITE FLOUR |
|---|---|---|
| Protein, gram | 12 5 | 10 1 |
| Fat, gram | 1 4 | 0 9 |
| Minerals, gram | 1 1 | 0 4 |
| Calcium, mgm | 44 0 | 20 0 |
| Phosphorus, mgm | 180 0 | 92 0 |
| Iron, mgm | 3 3 | 1 0 |
| Carotene, mgm | 0 2 | nil |
| Riboflavin, mgm | 0 02 | 0 01 |
| Thiamin, international units | 100 | 10–15 |

During wars, populations are forced to depend primarily on vegetable foods and wheat becomes of special importance There are many reasons why this is so

1 Soldiers need more food than they would require as civilians and their nutrition is of critical importance They get first choice of "protective foods" Workers in war industries also require more food

2 Conversion of vegetable foods into animal products or "protective foods" is a very costly process Only 5 to 10 per cent of the feed fed is recovered as meat and 15 to 20 per cent as milk or eggs

3 Our Allies, England and Russia, must eat the vegetable foods they raise, and some of the "protective foods" necessary to supplement their diets must come from this country

4 Wars are destructive both of foods and food producing areas

5 Efficient farming in war time is impossible because of diversion of manpower from the farm to fighting forces and war industries

6 Populations of occupied countries and prisoners must be fed

The United States started the war with a tremendous surplus of food, especially

of cereals The farmers were urged to increase the production of meat, milk and eggs, and as an inducement the government set prices on these "protective foods" high enough so the farmers could make a profit The livestock population reached an all time high and produced animal foods in greater quantities than ever before Yet there was not enough meat, milk and eggs produced to satisfy the demand with the result that rationing, both voluntary and governmental, had to be resorted to to spread the supply The surplus animal feeds were soon exhausted Liquidation of the livestock population was instituted to bring in line the animal population with available feed supplies The supplies of avail able "protective foods' are bound to decrease while at the same time demands are increasing The reduction of available "protective foods" has in fact been confirmed by the President of the United States (115) The decreased intake of "protective foods" must result in an increased consumption of bread This is characteristic of war food economy Under such conditions the character of the bread, whether white or whole wheat, becomes of importance in the national food economy and in national health

Dietary surveys before the war have been interpreted to show that large sections of our population are undernourished (5) If such a condition existed with a practically unlimited supply of "protective foods," their decreased intake must obviously cause a deterioration of the nutritional status of the population If the loss of "protective foods" of animal origin could be replaced by a food of plant origin equally 'protective," the nutritive status of the population could be maintained Fortunately in whole wheat we have just such a food Since the government has reduced the intake of the "protective foods' of the popula tion by rationing and their removal from civilian food channels the government bears a responsibility to the people to correct this possible dietary deficiency It has not chosen to substitute whole wheat for the "protective foods" lost, but instead has resorted to the use of "enriched' white flour

Under normal circumstances in a free country, the citizen has the right to choose a deficient diet if he wishes and to deal with the consequences in his own way In war, the government deprives the citizen of freedom to choose foods which he has learned from experience keep him in a fair state of health Under such conditions the government bears a responsibility to the citizen to help him adjust himself to changing food conditions so he may properly nourish himself The purpose of this review is to consider this whole question in the light of avail able evidence Special attention is paid to experiences in previous wars because of the light they shed on our present situation

Wars and nutrition Wars stimulate interest in nutrition The soldier must be fed properly to win battles The population at home must be fed adequately to maintain morale and to manufacture the munitions of war Nutrition has been the concern of military men during war or in anticipation of war

About 1880 beriberi had become a scourge among Japanese sailors, and fearful of the consequences of incapacitated naval personnel should war break out Takaki (6) investigated the problem, and found that the disease could be prac tically eliminated by substituting barley for a large part of the polished rice in the

diet of the sailors, increasing the meat and vegetables, and introducing condensed milk

J B Orr (7) recently recalled that during the Napoleonic wars the men from northern England and southern Scotland who lived in the country and had plenty of whole wheat grain, milk, eggs and vegetables were big, powerful and energetic men who made the best infantry soldiers of Europe During the Boer war a large percentage of the recruits from this district were short frail weaklings who could not be used as soldiers A commission was appointed to investigate the cause of this striking change in the physical condition of these men, and the most probable explanation found was that many people had moved off the land and had gone into the slums of the big cities where their eating habits had changed, and they were depending too largely on white flour and sugar

Wiley (8) pointed out in 1915 that recruiting officers in France, Germany, England and Italy were struck with the growing percentage of men unfit for military service, and the generally accepted cause was deficient nutrition, especially in infancy and childhood Drummond (2) pointed out that the Italian disaster at Caporetto was in large part due to "more than 7 months' subsistence on inadequate nourishment "

McCarrison (9) after the last war emphasized the rôle of nutrition in gastrointestinal disturbances and emphasized the importance of vitamin B (vitamin B complex had then not been differentiated) He insisted that bad diets consisting of white bread, sugars, margarine devoid of the A vitamin, boiled vegetables and tinned meat are the foundations of gastrointestinal disease (10)

In the present war, gastrointestinal disorders are receiving a great deal of attention in England Gastrointestinal disorders and functional dyspepsias outclassed in incidence any other group of disorders (11) and accounted for 25 per cent of the cases sent home from the British army in France in 1940 Apparently the disorders of the stomach and duodenum had become more common in medical practice in England during the last few years (11) In very few instances have the gastric disorders originated during military service (12) Apparently most of the patients during civil life had learned to adjust their diets and mode of life to keep their indigestion under control Many of the acute ulcers were the result of breakdown of old standing ulcers under the conditions of active service (13) Under the strain of heavy air raids there was also an increase in perforated ulcers in the civil population (14)

The vitamin B complex has been reported in clinical studies (15) to be beneficial in gastrointestinal malfunction In experimental animals gastrointestinal disturbances are associated with deficiencies of the vitamin B complex or its various components (16, 17, 18)

THE RÔLE OF BREAD IN NUTRITION One of the best treatises on bread is that of Sylvester Graham (1) published in 1837 It is not only very interesting but is applicable to our present day bread problems In general, his ideas have proven to be scientifically correct His work is frequently quoted but apparently seldom read, and for this reason will be discussed here in some detail

Graham (1) emphasized that bread is one of the most important, if not the

most important, articles of diet entering into the food of man He also emphasized palatability in bread, that from the same wheat kernel can be prepared a most delicious article of diet or "the most miserable trash that can be imagined "

Graham (1) had an unusually good understanding of the nutritive properties of bread The whole wheat bread was almost a complete food and in addition could cure digestive disorders such as constipation and diarrhea He warned that the removal of the bran by bolting the milled flour reduced the nutritive value of the "fine flour" obtained

He (1) quoted a Judge Peters "Baron Steuben has often told me that the peculiar healthfulness of the Prussian soldiers was in a great measure to be attributed to their 'ammunition,'—bread, made of grain, triturated or ground, but not bolted, which was accounted the most wholesome and nutritious part of their rations "

It is of great interest that the vigor of the German soldier today has also been attributed by Wilder (19) to be due in a large measure to the same type of bread. "The world has been impressed by the terrific might of the German armies We have witnessed unheard of military efficiency The German soldier seems to possess unusual capacities Not only has he been able to endure extreme exertion but his poise and fortitude has been remarkable—Hitler and his henchmen would have us believe that this implies race superiority I don't believe it —But Germany early put to work the science of nutrition The German youth and the German army are well fed Their bread, Koumissbrot, part rye, part wheat, is made of whole grain flour—the ration calls for a pound and one half a day '

Similarly, Williams and Wilder (20) attribute much of the power of the Russian Army to their whole grain bread "The Russian army is fed whole grain Thus far it is the only army to match successfully the whole-grain eating army of the Nazis The endurance of the Russian citizen equals the vigor of the Russian soldier The Russian people, eating whole grain bread receive important nutrients denied to people who depend on ordinary white flour for their bread " Yet these authorities did not recommend whole grain for our army or our civil population

During wars, foods become scarce and bread assumes very great importance in the life of warring nations During the war between England and France near the close of the eighteenth century, wheat became very scarce in England Since her experience then has been repeated in World War I and again during this war, it will be described in detail as a quotation by a Mr Prior (1)

William Pitt was then prime minister of state and at his instance the government recommended to the people generally throughout Great Britain to substitute potatoes and rice as far as possible for bread in order to save the wheat for the foreign army This recommendation was promptly complied with by many of the people But still the scarcity was alarmingly great In this emergency parliament passed a law (to take effect for two years) that the army at home should be supplied with bread made from unbolted wheat meal, solely for the purpose of making the wheat go as far as possible —My father whom I have often heard talk these things over was a miller and baker and resided in the county of Essex on the border of Suffolk and near the barracks containing eighty thousand soldiers

He contracted with government, to supply the eastern district of the county of Essex, with the kind of bread I have mentioned and he used always to send me with it to the depositories on the day it was baked and though I was then a youth, I can still very distinctly remember the angry looks and remarks of the soldiers, when they were first supplied with it Indeed they often threw their loaves at me as I passed along, and accompanied them with a volley of curses The result of this experiment was, that not only the wheat was made to go much farther, but the health of the soldiers improved so much and so manifestly, in the course of a few months, that it became a matter of common remark among themselves, and of observation and surprise among the officers and physicians of the army These gentlemen at length came out with confidence and zeal on the subject, and publicly declared that the soldiers were never before so healthy and robust, and that disease of every kind had almost entirely disappeared from the army The public papers were for months filled with recommendations of this bread, and the civic physicians almost universally throughout Great Britain pronounced it far the most healthy bread that could be eaten, and as such, recommended it to all the people, who very extensively followed the advice —and the coarse wheaten bread was very generally introduced into families—female boarding schools, and indeed all public institutions The nobility also generally used it, and in fact, in many towns, it was a rare thing to meet with a piece of fine flour bread The physicians generally asserted that this wheaten bread was the very best thing that could be taken into the human stomach, to promote digestion and peristaltic action, and that it, more than anything else, would assist the stomach in digesting other things which were less easily digested, and therefore they recommend that a portion of it should be eaten at every meal with other food

Still, after this extensive experiment had been made with such happy results, and after so general and full a testimony had been given in favor of the coarse wheaten bread, when large supplies of superfine flour came in from America, and the crops at home were abundant, and the act of parliament in relation to the army became extinct, most of the people who had before been accustomed to the use of fine flour bread, now by degrees returned again to their old habits of eating fine bread Many of the nobility, however, continued to use the coarse bread for a number of years afterwards General Hanoward, Squire Western, Squire Hanbury and others living near my father's, continued to use the bread for a long time, and some of them still used it when I left home and came to America, in 1816

In the war of 1914–18, England had a similar experience with shortage of food and wheat again became something precious as an article of diet The situation was thus expressed by Hopkins (21), "The present shortage in the food supply of the world makes important every detail of knowledge concerning human nutrition —Particularly desirable now is every scrap of knowledge concerning cereals Except in Arctic climates, bread and cereals are always important items in the food of mankind, and except where wealth has accumulated and luxury comes in its train, they are by far the most important Another question arises in connexion with grain foods which are of practical importance at the present time, what is the effect of increasing the percentage extraction of grain on the nutritve value of flour and bread "

In the United States the food situation was so alarming that physiologists drew upon extensive investigations on undernutrition to show that the population could well reduce their weight so that the basal heat requirements would be reduced In this way less food would be required and important food economies could be made Lusk wrote (22) "one may reduce the basal requirement of energy by undernutrition, and that this process may largely economize food"— "It may become necessary for Americans to 'train down hard' as people in the

warring nations of Europe are forced to do" In a similar vein Roth (23) wrote "The reduction of diet can safely include all principles, proteins as well as carbohydrates and fats"—"Many would be benefited by dropping occasionally or regularly one meal a day" In fact, loss of weight under some conditions was even considered beneficial "According to the statistics of life insurance companies a loss of 10 pounds from the average weight of a man of 55 years of age would result in an increase of his value as a life insurance risk' (24)

WAR BREAD—WORLD WAR, 1914–1918 *The Danish experience* The Allied blockade in 1917 cut off imports into Denmark upon which she depended to feed her large livestock population Hinhede (25) and Mollgaard were given the task of rationing the food for the country during the blockade The pigs, competing with the human for the same foods, were killed off The production of brandy and whiskey was stopped to conserve grains Rye, which formed the basis of the bread, was milled to 100 per cent and mixed up to 50 per cent with wheat bran which was taken from the cows Barley, milled to 95 per cent, was also added to bread "Some doctors were angry and wrote that Hinhede put the people on pig food and hen food" (25) In spite of the high fibre content of the bread, the digestibility was reduced only from 94 to 85 per cent (26) and they had available more than twice the amount of bread than they would have had had they milled their grain to 70 per cent. People did not complain nor were there any digestive troubles (26) as a result of the large amount of fibre in the bread The reason for the success of this program was the ability of the Danish people to make the whole rye-wheat bran mixture into a bread of good quality and palatability Hinhede (26) pointed out that the Danes had a hundred years of experience in making bread of good quality from such materials. The principal foods of the Danes during this period were this bran bread, barley porridge, potatoes, greens, milk and some butter The state of health of the people improved Thus this large scale war experiment proved that wheat bran is a good food.

*The English experience* As a result of submarine warfare, England faced a critical food shortage, and to extend their food supply the English had to mill their wheat to a greater percentage, first to 80 per cent and then to 90 per cent The English were not so fortunate as the Danes since they did not appear to have sufficient experience to bake bread of good quality and palatability with high extraction flours With characteristic thoroughness the English investigated *a*, the quality of the war bread *b* complaints made against the dark bread, and *c*, the nutritional value of the bread its palatability and its effect on health

*a, The quality of the war bread* The year 1917 when war bread was introduced was a hot year with the result that the war bread was frequently infected with a microörganism resulting in a condition known as "rope" (27) This "ropy" condition was probably favored by the higher nutritive value of the bread, since microörganisms just as human beings, thrive better on foods of higher nutritional value War bread contained more moisture than bread of 70 per cent extraction and the added moisture probably also favored the infection of the bread with "rope" This condition was investigated and much information was gained on preventing it

Because of inexperience in baking bread with flour of 90 per cent extraction, the war bread often lacked porosity and tended to be soggy and doughy and therefore less digestible Toasting was recommended to make the bread more digestible (28, 29) With experience the quality of the bread improved, but the English did not seem to be able to equal the quality of the Danish bread

b *The complaints* The complaints against war bread were investigated and Lord Rhondda, the Food Controller, discussed them in the House of Lords (30) Some of the complaints involved digestive disturbances in which any form of bread might be harmful "Roughly one half of the complaints refer to diseases the course of which cannot possibly be influenced by the kind of bread consumed" "The complaints as a whole largely reflect the fact that the nation is now nearing the end of the third year of unprecedented strain" Discomfort of the hot weather experienced in June was laid to the war bread (30) A feeling of flatulence or fullness was a common complaint (31) and this was ascribed to the soggy doughy bread, the result of inexperience in baking Looseness of the bowel was another complaint (31) and this was considered an advantage in a nation in which constipation was all too common Sufferers from digestive complaints tolerated the war bread well (33)

The dark color did not seem to be a frequent cause of complaint When properly baked the war bread was judged quite palatable and often preferred to the white breads (32)

In certain cases the government granted permits for the use of white flour, but only after careful investigation "In chronic functional disorders of the stomach (which make up the majority of cases of chronic dyspepsia) the pure wheaten flour can rarely be allowed, for the reason that such cases are so numerous and so protracted in their course that it would be impossible to meet the demands without seriously depleting the wheat supply of the rest of the population" (34)

c *The nutritional value of war bread, its palatability and effect on health* The Food (War) Committee of the Royal Society investigated the English war bread to answer the following questions (32)

1 What gain, if any, in food value to the nation will accrue from a rise in the milling standard from 80 to 90 per cent?

2 What would be the effect on the health of the population of the consumption of bread made from wheat flour of 90 per cent extraction?

3 How far would bread made from such flour prove acceptable to the population?

Digestibility studies were thoroughly and carefully carried out by competent and highly skilled scientists Three groups of 4 men in each group were the experimental subjects One group was studied under the supervision of F G Hopkins at the University of Cambridge, another, under Noel Paton at the University of Glasgow, and the third under the supervision of J A Gardner at the University of London (32) Two breads were compared, one from flour of 80 per cent extraction and the other from flour of 90 per cent The bread, with one exception, furnished from 45 to 60 per cent of the calories eaten which was more than the customary bread intake

A slight tendency to flatulence was observed at the beginning by some of the subjects, this later decreased and disappeared Looseness of the bowels was noted on the 90 per cent bread The feces always increased in bulk on the 90 per cent bread compared to the 80 per cent Some of the results obtained are given in table 2

The losses of energy and nitrogen of the high extraction bread were very small The gain was roughly 10 per cent in energy and 20 per cent in protein, even when allowance was made for feeding the 10 per cent offal to pigs

These investigators pointed out that it was difficult to determine the relative contribution of the food and of the gut to the feces since "the greater part of what leaves the intestine is not the undigested residuum of the food eaten, but material produced in the gut itself" This conception is of the greatest importance, since the nutritive quality of whole wheat and wheat bran was completely misunderstood as a result of digestibility experiments The larger amount of feces formed in animals fed bran or whole wheat was looked upon as unabsorbed food, leading to the common notion that wheat bran has little or no nutritive value for man This study directed attention to the possibility that the bulk of the feces did not represent undigested wheat bran but consisted largely of

TABLE 2

| | 80 PER CENT BREAD | 90 PER CENT BREAD |
|---|---|---|
| Av per cent utilization of energy | 96 14 | 94 5 |
| Av per cent utilization of nitrogen | 89 4 | 87 3 |
| Av weight of dry feces gram | 156 1 | 245 0 |

unabsorbed residues of material produced in the gut This idea has been fully proven experimentally (35)

The palatability of the war bread was studied with factory workers, 39 men and 8 women Of these, 20 men and 7 women, all volunteers, ate the experimental bread to the extent of 50 to 60 per cent of their calories for eight weeks The bread was also made available to other employees Educated sections of the employees such as managers and foremen were the majority of those who completed the test. The subjects who completed the test agreed the bread was palatable, easily digested and they did not tire of it When the bread was badly baked the people grumbled. After the experiment was over 61 of the employees signed a petition asking to have the bread made continually available

There was marked absence of flatulence and complete absence of diarrhea in the case of all who used the bread The feces in all cases were soft and passed without discomfort One of the subjects was cured of an acute diarrhea with pain, another of chronic constipation and a third of severe constipation

The bread was tried out among patients suffering from pulmonary tuberculosis, patients "notoriously capricious in their appetites and easily upset on improper food" The group consisted of 12 men and 13 women who ate the bread for 3 weeks The women consumed an average of 10 5 oz of the bread daily and the

men 11 8 oz The bread was preferred by the majority of patients, all of whom had more or less impaired digestions No evidence of digestive disturbance was noted in any single case All the women expressed preference for the bread after using it 3 weeks Of the men, 8 preferred the special bread, 2 had no preference and 3 either disliked it or preferred ordinary bread Of the patients that disliked it, all had capricious appetites and appeared to tire of it Two were febrile cases

The war bread in England was not an unqualified success, largely because of inexperience in making a good quality palatable loaf, but the nutritive superiority of the bread made from flour of 90 per cent extraction was so impressive that there was some question about continuing such a loaf Popular demand for the white loaf was, however, great, largely as a result of the unpalatable nature of so much of the war bread the people were forced to eat The food ministry allowed the return of the white loaf on the grounds that the "offal," or mill feeds, was necessary for the livestock (36) Lancet summarized the situation "Better bread could have been made with better knowledge, but we deprecate the use of the phrase 'better bread' to describe the return to white flour Those with a taste for such bread may consider it 'nicer,' but it is elevating personal predilection on a pedestal to make it the standard of goodness We still read in old books of one man wearing a 'better' coat than another, the words having no relationship to the warmth or the wearing quality of the coat and in this sense perhaps 'better bread' may stand Of course the *economic* necessity of the transitional period between war and peace may dictate the surrender of 18,000 tons of 'offal' weekly to farmers, pig-keepers and others in order to feed animal stock "

The experience of the United States with war bread was of too short duration to obtain a popular reaction to war bread Taylor (37) opposed bread from whole wheat flour on the grounds that "the function of the food administration is to secure and conserve food, not treat pre-existing diseases in a compulsory manner, applied to the majority who are not afflicted, as well as the minority who may be distressed, but still possess the right to select their treatment " The current introduction of "enriched bread" (5) involves the rejection of this notion and the acceptance on the part of the government of responsibility for the nutritive quality of the food of the people and the use of the Federal authority, at least in war time, to improve the nutritive level of the population whether they like it or not

The French had a disagreeable experience with war bread, largely because of the poor quality of the flour used and inexperience in baking a good quality loaf Apparently the millers did not always co-operate since they sometimes left in the flour "much dust, mildewed and foreign grains, ergot and dodder seeds" (38)

The population in Switzerland accepted their war bread of 87 per cent extraction without too much complaint Spriggs (31) quoted Professor Feer "In Switzerland only neurasthenics and hypochondriacs complain " This was probably exaggerated In war there is apt to be impatience with grumbling even for good causes Of interest, however, was the experience that hand-fed children

from the third month of age who had white flour in their formulas suffered no digestive disturbance when whole wheat flour replaced the white flour (31)

In Germany the war bread was sour and soggy (26) but the trouble there was that there was not enough of it. Rubner conducted extensive digestibility experiments with various breads and insisted on the basis of fecal losses that highly milled flours were most efficient. His work has been reviewed by Lusk (39). The work involved digestibility studies with special emphasis on the losses of nutrients in feces. The German outlook was analytical and they lacked sound nutritional concepts, yet Lusk (39), who either did not read the English work (32) or did not appreciate it, wrote "The American faddist who would have incorporated into the permanent law of the land the compulsory production of whole wheat bread and its infliction upon the population meets here his nemesis. The British people during the war felt that whatever else happened, they would return to white bread as soon as they possibly could. Here instinct, supported by Science, triumphs once again over ignorance and bigotry." Science has not supported this contention of Lusk but has completely disproved it. The composition of feces was an insufficient basis for judging the nutritive value of bread or any other food.

Out of the war came the first thorough-going study by Osborne and Mendel (3) of the nutritive value of the wheat kernel, with the rat as the experimental animal. They established the following

1 The proteins of white flour are inferior to those of the wheat germ or wheat bran

2 The vitamins are associated with the proteins of high quality in the wheat germ and wheat bran

3 Meat, milk or eggs when furnishing one-third of the proteins consumed will adequately supplement the white flour proteins

As our knowledge of nutrition advanced, especially with a better appreciation for the rôle played by proteins of high quality, minerals and vitamins, our understanding of the effect of milling on the nutritive properties of wheat increased

For thousands of years wheat and rice have figured prominently in the diets of the bulk of the human population. The whole grain cereals provided adequate amounts of the vitamin B complex, proteins of good quality, minerals and other essential factors such as vitamin E, carotene, the essential unsaturated fatty acids, and perhaps many as yet unidentified factors. The whole grain cereals needed supplementation with either meat (including some bone), milk or fish and some fruits or vegetables. Apparently this could readily be accomplished. With the advent of modern milling processes, the germ and outer coatings of the wheat berry are removed from the flour. With these "offals" or by products are lost the bulk of the vitamin B complex, most of the important minerals including iron and calcium, most of the fat including vitamin E and a larger part of the protein of high biological value, leaving in the white flour mostly carbohydrates and proteins of lowered quality (3). The wheat was milled in this way because people wanted white flour, and because of the greater stability of white flour toward rancidity changes after the removal of the fat. Moreover,

insect pests such as the flour weevil did not thrive so well in white flour as in whole wheat flour because of the removal of nutrients required by the flour weevil (40) Instead of being alarmed at the decreased nutritive value of white flour as shown by the inability of insect pests to thrive on it, the production of white flour was hailed as a great forward step Nutrition at the time had not progressed to the point where the true nature of the phenomena observed was fully appreciated During the intervening years, the high nutritive qualities of whole wheat bread became increasingly apparent as the evergrowing number of factors resident in the whole wheat became known The nutritional poverty of white bread as compared with whole wheat bread was so great as to demand attention under the impact of war when it was realized that the nutrition of the army and civilian population would play a major rôle in the struggle for survival The bread question became prominent both in England and America and received a lot of attention

WORLD WAR II, 1939– *The English experience with fortification of white bread* The discussion on fortification of white bread in England was opened by Mr Boothby (41), parliamentary secretary to the Ministry of Food, when he announced in parliament that white flour was to be fortified with thiamine The reason he gave for fortifying the white flour was to meet the objection against white flour by nutrition experts, who stressed the superior nutritive value of whole wheat flour as compared with white flour The government had not suspended the manufacture of white flour because the great majority of consumers preferred white bread and because the keeping qualities of white flour were definitely greater than those of whole meal flour "To overcome the objection that white flour was lacking in vitamin content, it had been decided to fortify it with vitamin $B_1$" (41) Whole wheat bread was to be available at the same price as white bread

This program was endorsed by Moran and Drummond (42) who pointed out as an additional objection to whole wheat bread that it would reduce the available wheat by-products for the stock industry and "in particular, jeopardize our milk supplies" In their paper they stated, "The introduction of the new white flour will undoubtedly stultify the controversy of white versus brown bread, since it is mainly in respect of vitamin $B_1$ that the white loaf has been open to attack There is not the same evidence that we are deficient in vitamin E or members of the vitamin $B_2$ complex which are present in whole meal flour " In this he proved to be wrong because the controversy became more intense than ever

A leading article in Lancet (43) reviewed the whole question and gave the following arguments in favor of fortifying white flour with thiamin

1 People do not like brown bread but pigs and chickens like and need the "milling offals" when white flour is made

2 Brown bread needs much more yeast to bake than white bread

3 Brown bread has usually been more expensive than white

4 Under war conditions involving storage problems, white flour is preferable to whole wheat flour because it keeps better

5 By ordering that whole wheat bread shall be available at the same price as white, the government removed the grievance of those who complain that they do not eat brown bread because it is too expensive

6 By the addition of thiamin to white flour, the most serious vitamin deficiency of white flour is made good

7 The average daily bread intake in England was 0 5 pound per capita The consumption was however unevenly distributed, better-off people averaging about 4 ounces a day and working people as high as 1 5 pounds It is these working people with low intake of the expensive "protective foods" such as milk, cheese, eggs, fruits and vegetables whose diet will be "highest in carbohydrate, and who will most benefit by fortification with added vitamin" (42)

Thus was ushered in a revolutionary change in human life—the acceptance by government of responsibility for the nutritional welfare of the population It was unfortunate that with respect to the bread, the food staple of the common people, the program started with the uncertain assumption that by adding thiamine to white bread, its nutritive value when combined with the remainder of the diet available to the common people, has been restored to that of whole wheat flour This necessitated the assumption, based on no evidence, that the English diet which included white bread as the staple food was deficient only in thiamine

The program of fortifying white bread with thiamine did not go unchallenged Sir Graham Little (44) pointed out that it was proved by statistics that the health of the nation during the last part of the great war and in the period that followed was better than it had been before and it was attributed in large part to the whole wheat bread the English were obliged to eat as a result of the war

The Medical Research Council in a special memorandum (45) immediately called attention to the importance of the vitamin $B_2$ complex in whole wheat bread, and that it could not be ignored even though the knowledge on this subject is less exact than for vitamin $B_1$ The memorandum also called attention to the loss from white flour of important fat soluble vitamins, minerals, and protein of high quality They recommended under milling, which would result in the extraction of 85 per cent of flour from wheat, in place of the usual 72 per cent, thereby saving the bulk of the nutritive value of the wheat berry without including any appreciable amount of the rough bran

Thus the controversy of white bread versus brown bread was started and it was discussed in the *Times*, in the trade journals and medical journals The discussion became intense, bitter and acrimonious The nutritional aspects of the discussion were well mirrored in Lancet, and will be discussed in detail because of the great importance it was bound to have on nutritional thought.

In a second leading article in Lancet under the heading "Second thoughts on bread" (46), the discussion was still largely on the basis that the thiamine content of the wheat berry is the factor to be concerned with in the choice between white flour and whole wheat flour

Graham Little (47) attacked the thiamine fortification program and considered

the minister's objections to whole wheat bread in the light of the nation's experience in the last war He discussed

1 *Alleged indigestibility of whole wheat bread* Graham-Little referred to the report of the Committee of the Royal Society "On the digestibility of bread" (32) This report showed that there were no symptoms of indigestibility on whole wheat bread in healthy subjects or even in invalids

2 *Alleged unpalatability* Only a small minority, 8 per cent, expressed a preference for ordinary bread after a trial was made of both breads The "invincible preference" for white bread is a myth

3 *Keeping qualities* He questioned whether the difference in keeping quality is such a great factor as to prohibit the use of whole wheat flour

He suggested that the resistance to whole wheat bread may come from the flour milling industry, a giant trust, who were safeguarding their 100 million dollar investment in milling equipment

In Parliament (48) it was brought out by a question from Fremantle that all medical men consider whole wheat bread "infinitely better" than fortified bread The parliamentary secretary replied that the really primary reason for the fortification of white bread with thiamine was the large number of people who did not care to eat brown bread

Graham-Little (49) attacked political considerations which result in approval by default and quotes from the Penquin Book, *Science in war* on advisory bodies, "They seem to have been much more concerned with agreeing with government decisions than with challenging them in the name of science In the long run the 'yes-men' of science are likely to be at least as dangerous as the 'yes-men' of politics "

Franklin Bicknell (50) urged the parliamentary minister, "If the minister knows, as he must, that stone-ground flour is best for the nation he should say that $B_1$ in bread will help slightly, but that he cannot provide really valuable stone-ground bread because of the millers "

In a survey by the Ministry of Food's scientific adviser to determine the relative popularity of brown and white bread with the public (51) the following information was brought out In the poorer areas, 41 to 46 per cent of the public ate some quantity of brown bread regularly and in the richer areas the figure was 65 per cent Brown bread was actively disliked in about 34 per cent of cases in all classes If brown and white bread were the same price about 28 per cent of the poorer people and 13 per cent of the richer people would buy more brown bread

Added impetus was given to the controversy when Chick (52) showed by feeding experiments with rats that white flour enriched with thiamine was decidedly inferior to whole wheat flour The rats on whole wheat flour gained about twice as much weight as those on "enriched" white flour, and they utilized their food more efficiently The rats on the whole wheat flour diet made 1 gram of gain with a food intake of 2 47 grams while it required 3 02 grams of the "enriched" white flour diet to make the same gain When the diets were switched, the rats transferred from the "enriched" white flour diet to the whole wheat

diet made an immediate spurt in growth while those transferred from the whole wheat flour diet to the "enriched" white flour diet suffered an immediate check in growth

A leading article in Lancet (53) immediately took cognizance of this new information It pointed out that considerations of the inferiority of white flour can no longer be concerned only with vitamin $B_1$ but with other factors, the nature of which was not very certain Lancet now took the stand that the "sign post points to whole meal as the high road to better health and greater food economy" "Political or economic considerations may make it impossible to take this road in wartime, but the general conclusion from the nutritional stand point is not thereby affected"

Graham Little (54) inquired of the food minister in Parliament whether Doctor Chick's work had any influence on the policy to fortify white bread with thiamine and the answer was in the negative

As a result of the controversy to date the British Government modified its bread policy which Lord Woolton (55) announced in the House of Lords He had been impressed by the unanimity of scientific opinion on the nutritive value

TABLE 3

| FLOUR | FIBRE | ASH | PROTEIN | VITAMIN $B_1$ | CALCIUM | PHOSPHORUS |
|---|---|---|---|---|---|---|
| | *per cent* | *per cent* | *per cent* | *international units per gram* | *mgm per 100 gram* | *mgm per 100 gram* |
| I 85% | 0 00 | 0 90 | 11 4 | 1 20 | 27 | 203 |
| II 85% | 0 85 | 0 94 | 11 5 | 1 15 | 27 | 211 |
| White, 73% | 0–0 2 | 0 46 | 10 6 | 0 35 | 15 | 101 |
| Wholemeal 100% | 1 8 | 1 51 | 11 9 | 1 40 | 36 | 343 |

of wholemeal bread He considered it the proper attitude of the government to make wholemeal or national wheatmeal (85 per cent extraction) available in adequate supply to the public at the same price as white bread, and at the same time to draw the attention of the public to the advantages of brown breads The government would at the same time continue its efforts to produce a white bread fortified with thiamine.

Willcox (56) pointed out that in Mesopotamia in the last war, a loaf made from 75 per cent whole wheat meal and 25 per cent white flour proved to be very palatable Wholemeal bread properly baked has more flavor than white bread and he did not consider the universal use of whole meal bread an insuperable problem

On May 22, 1941, the Medical Research Council (57) issued a second memorandum on national wheatmeal flour in which they defined more exactly the character of the 85 per cent wheat meal which they recommended (table 3)

They recommended flour I with only 0 6 per cent fibre Since that would require modification of milling machinery, they recommended the interim adoption of flour II containing not less than 1 international unit of thiamin (3 micro-

grams) per gram nor more than 0 9 per cent fibre, assuming a water content of 15 per cent They recommended the addition of calcium to neutralize the effect of phytic acid which diminishes the availability of the calcium present in the diet They opposed the addition of iron because of its possible destructive catalytic effects "On technical and aesthetic grounds, the loaf baked from such flour was excellent" In a leading article, Lancet (58) pointed out that the recommendation was the result of "a most careful scrutiny of the nutritive value of different parts of the wheat grain" They strongly recommended the bread but noted "Against the change can only be set popular prejudice, (much of it will go when everyone has sampled the national loaf) the better keeping qualities of white flour and an understandable reluctance to scrap the machinery which is now producing vitamin $B_1$ cheaply and on a large scale"

TABLE 4

| WHEAT IMPORTS | HUMAN FOOD | | AVAILABLE CALORIES | DIGESTIBLE PROTEIN | AVAILABLE Calcium | AVAILABLE Iron | THIAMIN | RIBOFLAVIN | NICOTINIC ACID |
|---|---|---|---|---|---|---|---|---|---|
| $10^6$ *tons* | | $10^6$ *tons* | $10^{12}$ | $10^6$ *tons* | *tons* | *tons* | $10^{12}$ *i u* | *tons* | *tons* |
| 6 | Flour, 75% | 4 5 | 16 11 | 0 45 | 450 | 58 5 | 2 025 | 2 25 | 67 5 |
| | Milk from 1 5 × $10^6$ tons wheat feed | 3 43 | 2 23 | 0 109 | 4,120 | 4 1 | 0 79 | 5 15 | 15 1 |
| | Total | | 18 34 | 0 559 | 4,570 | 62 6 | 2 815 | 7 40 | 82 6 |
| 5 3 | Flour, 85% | 4 5 | 14 22 | 0 446 | −45 | 90 | 4 05 | 4 50 | 203 |
| | Milk from 0 8 × $10^6$ tons wheat feed | 1 83 | 1 19 | 0 058 | 2,200 | 2 2 | 0 42 | 2 75 | 8 1 |
| | Total | | 15 41 | 0 504 | 2,155 | 92 2 | 4 47 | 7 25 | 211 1 |
| Change in total nutrients available with 700,000 tons shipping space saved | | | −2 93 | −0 055 | −2,145 | +29 6 | +1 65 | −0 15 | +128 5 |

The proponents of fortification opposed the national wheatmeal (85 per cent extraction) on grounds of non-acceptability by the British public and because the change over from a 75 per cent extraction of the wheat berry to an 85 per cent would so sharply reduce milk supplies as a consequence of the loss of wheat feed for the cattle, that there would be a net loss in important nutrients such as digestible protein, riboflavin and available calcium This thesis was first presented in great detail by Wright (59) and was subsequently critically reviewed by Bacharach (60) who corrected some minor errors in Wright's paper (59), but reached essentially the same conclusion the details of which are given in table 4

The following conclusions were drawn from the table

1 There was a saving of 700,000 tons of shipping space when the same amount of flour, namely, 4,500,000 tons, is obtained by milling wheat to 85 per cent instead of 75 per cent

2 The loss of milk as a result of the decreased wheat feed for cattle added to the lower digestibility of 85 per cent flour (digestible protein—white flour, 91 per cent, national wheatmeal, 86 per cent, digestible carbohydrates—white flour, 94 per cent, national wheatmeal, 89 per cent) would result in the following changes

*a* Loss of 55,000 tons of digestible protein
*b* Loss of 2,145 tons of available calcium
*c* Gain of 1 65 $\times$ $10^{12}$ I U of thiamin
*d* Gain of 128 5 tons of nicotinic acid
*e* Gain of 29 6 tons of available iron
*f* Loss of 2 93 $\times$ $10^{12}$ of available calories
*g* Loss of 0 15 ton of riboflavin

Kent-Jones (63) weighed the gains against the losses, and urged that the losses of digestible protein, calcium and riboflavin far overbalanced the gains of thiamin, iron and nicotinic acid The gain in nicotinic acid he argued is of no importance since the average diet already has more than adequate amounts The gain in thiamin is also of no importance since white flour was to be fortified with thiamin He concluded "the case for national wheatmeal is finally and definitely exploded on sound scientific grounds"

Fraenkel (64) criticized Bacharach's table (60) on the ground that he jumbled shipping space with nutritional values If shipping space were the prime consideration, it would be useless to talk about nutritional values If nutritional values were concerned, the whole question must be put on a nutritional basis If 6,000,000 tons of wheat were milled to 75 per cent, 4,500,000 tons of white flour would be obtained plus 1,500,000 wheat feed ("offal") By milling to 85 per cent, 5,100,000 tons of wheatmeal flour would be obtained and only 900,000 tons of wheat feed Fraenkel pointed out that for proper consideration, only 4,500 000 tons of 85 per cent wheatmeal flour should be milled from 6,000,000 tons of wheat leaving 1,500,000 tons of wheat feed "offals" and unmilled wheat combined to be fed to cattle In this way, the same amount of milk would be obtained regardless of the flour produced and no shipping space would be involved The difference in nutrients available between milling 75 per cent white flour or 85 per cent wheatmeal would be the result of the difference in digestibility of 75 per cent flour and 85 per cent wheatmeal Using Bacharach's data, Fraenkel recalculated the changes in nutrients from the change over to 85 per cent wheatmeal In each case, 4,500 000 tons of flour would be obtained and the same amount of milk, namely, 3,430,000 tons

On this basis, as a result of the change over from 75 per cent to 85 per cent flour, there would be little change in digestible protein, the loss of available calcium would be cut down, and there would be no loss but a gain in riboflavin The gains in the other nutrients would be enhanced (table 5)

The argument against 85 per cent wheatmeal because of decreasing the milk supply apparently did not carry very much weight It was widely discussed pro and con E. Lester Smith (65) pointed out, "Dr Wright cannot have his bread and let the cow eat it" He favored 85 per cent wheatmeal to improve the

health of the population, and even favored if necessary the feeding of the white flour to cows to maintain the milk supply Lancet (66) pointed out that "the cow was not a 'penny-in-the-slot' machine into which you insert a production ration of any given size and automatically get back the exact equivalent of your feeding stuff as milk"

Chick's paper on the superior nutritional value of national wheatmeal over "enriched" white flour led the proponents of fortification of white flour to search for experimental evidence to support their case This was soon forthcoming in a paper by Wright (67) who criticized Chick's work (52) because the food intakes of the rats receiving "enriched" white flour and national wheatmeal were not equalized, the wheatmeal being consumed in greater quantity, thus accounting for their superior growth and well being Doctor Wright argued that 85 per cent wheatmeal was more palatable to rats than white flour, and since no way was known to equalize the palatability of the two flours, the rats receiving the national wheatmeal were fed only so much food as the rats on "enriched" white flour consumed Under these conditions there was little difference in

TABLE 5

| | AVAILABLE CALORIES | DIGESTIBLE PROTEIN | AVAILABLE | | THIAMIN | RIBOFLAVIN | NICOTINIC ACID |
|---|---|---|---|---|---|---|---|
| | | | Ca | Fe | | | |
| Change in total nutrients available resulting from a change to 85% flour from 75% flour | $-1\ 89 \times 10^{12}$ | −4,000 tons | −305 tons | +31 5 tons | $+2\ 025 \times 10^{12}$ I U | +2 25 tons | +135 5 tons |

growth of the rats The experiment was repeated with the two breads fed along with other foods so that the total would represent the ordinary mixed human diet The diet consisted of bread 35 5 per cent, boiled potato 30 0 per cent, cooked meat 6 0 per cent, fresh milk 3 0 per cent, cooked fish 3 0 per cent, margarine 3 0 per cent, cheese 0 7 per cent, cooked vegetables 14 0 per cent, jam 1.2 per cent, eggs 1 2 per cent and sugar 2 4 per cent On the mixed diet containing the national wheatmeal the rats were somewhat superior, even though limited to the same food intake voluntarily consumed by the rats on the "enriched" white flour diet However, her work was not confirmed by similar and more extensive studies (107) which instead supported Chick's work

Though the government (55) announced that national wheatmeal would be available in abundance, a hitch developed somewhere and it was not done The question was raised in Parliament (68) and Lancet (69) demanded an investigation to locate the obstruction, whether it was with the millers, master bakers or the public

During this period a very revealing American article on bread appeared Pewters, Mason and Higgins (70) compared whole wheat flour with "enriched" white flour when added to a basal diet which was representative of that of thou-

sands of Americans It included in addition to bread which was the only variable, butter, skim milk powder, cornflakes beef, potato, cheese, polished rice, gelatin, apple jelly, string beans, carrots, peaches, pears, sugar, candy and cake or cookies Thus, any results obtained would, in so far as the diet was concerned, be applicable to human beings Four types of flour were compared white flour, white flour fortified with thiamine, white flour fortified with thiamine and riboflavin and wholewheat flour The rats grow a little better with thiamine enriched flour than with white flour, a little better still with flour enriched with thiamine and riboflavin, but decidedly better on whole wheat flour Not only did the rats on the whole wheat flour grow more but their appearance was improved They concluded that flour must be enriched with factors other than thiamine and riboflavin to obtain a flour of nutritive quality comparable to that of whole wheat flour After some uncertainty in the interpretation of these data (102) the workers in this laboratory provided additional evidence (116) which showed conclusively that whole wheat bread is nutritionally superior to "enriched" white bread

TABLE 6

*Average intake of plentiful foods consumed in 1935 and 1941 in ounces per week*

| FOOD | WOMEN 1935 | WOMEN 1941 |
|---|---|---|
| Bread | 35 2 | 45 6 |
| Flour in puddings etc | 3 0 | 5 1 |
| Oatmeal | 0 5 | 1 6 |
| Proprietary cereals | 0 8 | 1 4 |
| Potatoes, cooked | 21 1 | 33 0 |
| Green vegetables, cooked | 9 2 | 13 0 |
| Root vegetables, cooked | 4 7 | 7 4 |
| Legumes cooked | 2 8 | 2 4 |

The importance of bread in the English dietary under wartime conditions was emphasised by a comparative study of middle-class diets in peace and war (71) as shown in table 6

Since so large a part of their food consisted of bread, the protective factors in the whole wheat bread were of great importance

The acceptance of the national wheatmeal bread was unsatisfactory and Lancet in a leading article (72) inquired into the causes It pointed out that "never before had the knowledge been sufficient to select with confidence a loaf which would look and taste almost like white bread and yet contain such a large share of the best nourishment of the whole wheat grain " The Medical Research Council had recommended the preparation of a bread by undermilling to 85 per cent which would give a uniform product of satisfactory appearance and taste The instructions of the Ministry of Food to the millers was so worded, however, as to allow the mixture of 15 per cent bran with white flour and still meet the specifications of the Food Ministry The result was lack of uniformity in the national wheatmeal flour which was responsible in part for its lack of

acceptance Lancet (72) observed "In this conflict between the national and individual interests, the millers have thus won the day, and a golden opportunity of furthering the health of the people was lost"

The character of the national wheatmeal loaf became the subject of a heated controversy In Parliament Profumo (73) asked the parliamentary secretary to the Ministry of Food on what grounds some of the recommendations of the Medical Research Council on the national wheatmeal loaf were turned down The parliamentary secretary answered that none of the recommendations were rejected, whereupon Profumo told the secretary he did not know what he was talking about

Graham-Little (74) suggested one of the reasons for bungling the program for an increase in consumption of long extraction flour is the influence exercised by the milling industry, with its formidable occupation of key positions in the Ministry of Food He urged the medical profession to lead the revolt against the Food Ministry

Graham-Little (75) pointed out that the recommendations of the Medical Research Council limiting the wholemeal flour to 0 9 per cent crude fibre with 1 0 international unit of thiamine per gram was largely ignored As a result, a variable and poor quality loaf was produced and in spite of the expenditure of £29,000 in advertising, the consumption of national wheatmeal was about 7 0 per cent He (75) sponsored a motion in the House of Parliament to appoint a committee to find out what are the considerations which obstruct the exclusive provision of a national wheatmeal loaf of the highest possible extraction "The point of the demand is that it is feared that the reasons operating against the production of this type of loaf are not based upon the science of nutrition but upon the interests of commercial firms"

The whole controversy ended on March 11, 1942, when Lord Woolton (76) announced in the House of Lords, "His Majesty's Government have therefore decided to increase to 85 per cent the ratio of flour from milled wheat in this country This means we shall stop the production of white bread" It was hoped the same bread would soon be supplied to the army The decision was welcomed Lord Hankey "hoped the millers and bakers would play their part in producing a palatable bread all over the country" "So the matter ended for the time with victory for science because it had an economic ally in shipping"

It should be emphasized (77) that the change to 85 per cent national wheatmeal flour was not the result of shipping considerations only, but saving of shipping space tilted the balance in favor of national wheatmeal

Leading articles in Lancet (78) and the British Medical Journal (79) greeted the decision with much satisfaction The British Medical Journal summarized the controversy, "Thus comes to an end a controversy that has lasted for 2½ years, and on which many hundreds of thousands of words have been spent in speech and print As the public accepted the standard loaf in the last war without much ado, it has been difficult for anyone outside the Ministry of Food to understand why the liberty of the subject in respect of white bread has been looked on as such a precious thing During the past three months as the columns

of the *Times* have shown, criticism of that Ministry's policy has become progressively acute, and Sir Ernest Graham Little has attacked it in the House of Commons, this time to good effect —There has been a suspicion in more than one quarter that the interests of the millers have been allowed to stand in the way of the nation's health, and until this moment the Ministry of Food has shown signs of falling short of whole-hearted advocacy of its wheatmeal loaf —It is a punishable offense to water milk and dilute the solids in it Why, then should it be thought praiseworthy to remove from the wheat berry the valuable minerals and vitamins it contains '

One of the results of the controversy was a reinvestigation of the digestibility of wholewheat bread and the biological value of the proteins of various parts of the wheat berry Drummond (80), acting as scientific adviser to the Ministry of Food, suggested that the branny layers of the wheat berry, even when finely ground, might be injurious to some gastrointestinal tracts A wheat flour was chosen which had removed from it the more indigestible fractions, but retained the bulk of its minerals and vitamins It was largely on digestibility trials that it was decided to go for an 85 per cent flour This was immediately challenged

TABLE 7

| EXTRACTION OF WHEAT | DATA USED BY WRIGHT | | DATA OF KREBS AND MELLANBY | |
|---|---|---|---|---|
| | Utilization | | | |
| | Protein | Calories | Protein | Calories |
| *per cent* | *per cent* | *per cent* | *per cent* | *per cent* |
| 75 | 89 | 92 | 91 | 97 |
| 85 | 81 | 87 | 89 | 94 |

by Scott (81) who insisted that "there is not a scrap of published evidence that an 85 per cent extraction flour is more digestible than whole wheat meal " He referred to Drummond's claim as outrageous

Krebs and Mellanby (82) reinvestigated the utilization of calories and protein of white flour and national wheatmeal and found the differences less than those quoted by Wright (59) who argued that the losses from decreased digestibility would balance the additional flour gained from an 85 per cent extraction, and that nothing would be gained by the change to 85 per cent wheatmeal from white flour (table 7)

On the basis of the figures of Krebs and Mellanby (82), there is little difference in digestibility (utilization) of white flour and 85 per cent extraction flour

A most revealing communication on this subject was published by Chick (83) who investigated by the growth method with rats the biological value of the proteins of whole wheat flour, 85 per cent extraction flour and white flour The proteins of 85 per cent extraction flour showed an advantage in growth of 13 to 16 per cent over the proteins of white flour, while whole wheat flour showed an advantage of 17 to 26 per cent The utilization (digestibility) of whole wheat flour and national wheatmeal was less by 6 per cent and 3 per cent respectively

Thus, the superior biological values of the proteins of the outer layers of the wheat berry more than offset the greater digestibility of the proteins of white flour

The investigations of Borgstrom (35) have done much to enable us to understand Chick's results (83) and have clearly shown how erroneous they were who held that wheat bran had little value or was worthless to the human because of its alleged indigestibility He (35) showed that *in vitro* as much as 90 per cent of the nitrogen of wheat bran could be brought in solution by the action of pepsin and trypsin The nitrogen could be removed from the intact cells by these enzymes without the necessity of rupturing them The larger part of the nitrogen in the feces of bran fed animals originated not in undigested wheat bran proteins, but in the digestive secretions which were stimulated above normal by the bran and in the bacteria This nitrogen exists in a state largely unavailable to the organism Wheat bran increased the fecal output as well as the peristalsis in the gut

The advantages of the increased quantity of digestive juices was an increase in the rate and intensity of digestion, thereby increasing the amount of nutrients released from the wheat bran for the nutrition of the organism These nutrients apparently compensated for the loss of fecal nitrogen arising from the failure of these digestive secretions to be reabsorbed with the result that the animal retained more nitrogen for physiologically productive purposes as shown by Chick (83) These results were obtained with rats and with men as the experimental subjects Even after about 60 per cent of the wheat bran was removed by previous in vitro digestion the residue was still able to reduce the loss of nitrogen in the urine

The calcium of the wheat bran was found to be readily utilizable (35) but it was apparently offset by the loss of calcium in the digestive secretions The alleged unavailability of the calcium of wheat bran can be explained by the losses of calcium in the digestive juices which fail to be reabsorbed

In the light of the evidence now available, the English have been too conservative in milling their wheat to 85 per cent and discarding the bulk of the bran The value of bran in the human diet has been established by the mass-experiment of Hinhede (26) in Denmark, and proven scientifically by the investigations of Osborne and Mendel (3), Borgstrom (35) and the Food (War) Committee of the Royal Society (32)

THE CANADIAN EXPERIENCE McHenry (84) in 1940 compared "enriched" white bread with whole wheat bread and found the latter greatly superior The Canadian medical men and nutritionists early appreciated the superiority of whole wheat bread over "enriched" white bread and their leading scientists, among them McHenry, Tisdall and Best (105) took a definite stand against fortification of white flour They insisted that the way to get the vitamin B complex in bread was to so mill the wheat that these vitamins would be retained The millers and scientists co-operated in extensive milling experiments with some interesting results They controlled their milling operations by thiamin analyses, on the assumption that if thiamin were retained, "the attendant members" of

the vitamin B complex would also be retained They found great variability in the thiamin content of their wheat

One of the interesting results of their investigations was the difference in the thiamin distribution in the wheat kernel By breaking the kernel transversely, they obtained three fractions, the germ with 3300 I U per pound, the germ end without the germ with 915 I U per pound and the brush end with 252 I U per pound

By decreasing the moisture content of the wheat milled from 15 5 per cent to 12 5 per cent, they could get a white flour with the retention of a fairly large proportion of the vitamins of the wheat berry As a result of a rather extensive series of investigations of these nutritionists in co-operation with the millers, a white flour of 75 per cent extraction was produced with 303 I U of thiamin per pound as compared with 254 I U in the white flour made in the ordinary milling process (15 5 per cent moisture) (105)

Taking advantage of these investigations the Canadian Government issued the following specifications for two types of flour and two types of bread

1 Vitamin B white flour shall contribute not less than 400 I U of vitamin $B_1$ "with the other members of the vitamin B complex in the quantities associated with this amount of vitamin $B_1$ in the wheat from which the flour was produced "

2 Vitamin B flour shall be flour other than white flour that will contribute per pound of flour not less than 550 I U. of vitamin $B_1$ together with the attendant members of the vitamin B complex

3 Vitamin B white bread shall be baked solely from vitamin B white flour with 4 0 per cent non fat milk solids and which shall contain not less than 220 I U of vitamin $B_1$ per pound together with the attendant members of the vitamin B complex

4 Vitamin B bread shall be bread made solely from vitamin B flour with not less than 4 0 per cent non fat milk solids and shall contain not less than 300 I U of vitamin $B_1$ per pound together with the attendant members of the vitamin B complex

THE AMERICAN EXPERIENCE. Compared to the way the English treated their bread problem the American performance seems below par Unlike the English, they have not discussed with any degree of thoroughness the relative merits of whole wheat bread, white bread and "enriched' white bread The whole question of fortification of foods including bread was first discussed at a symposium held at the meeting of the American Institute of Nutrition at Toronto, April 1939 Morgan (85) discussed the difficulties and uncertainties of the problem and warned that " 'Proceed with caution' must remain the watchword still " Her subsequent investigations (137) justified this cautious attitude since she found with rat feeding tests that whole wheat bread was superior to "enriched" white bread Moreover, because of the large intake of bread, whole wheat bread proved to be a slightly better source of the vitamin B complex than about 10 per cent yeast

Roberts (86) discussed the nature of the American diet and showed the multiplicity of food factors removed in the milling of wheat She cautioned that "if

the plan is accepted, the exploitation of the products should be so safeguarded as not to give the impression that all the deficiencies of the cereal have been overcome" She considered fortification a "first-aid" measure and said, "We must remember that the last word has not yet been spoken in respect to nutrition and that there may still be many essential dietary factors of which we are as yet unaware We cannot, therefore, trust solely to fortification, but must still put our major faith in a varied diet containing generous amounts of natural foods"

The most outspoken against fortification was Sebrell (87) who said, "Finally, to me it seems a little ridiculous to take a natural foodstuff in which the vitamins and minerals have been placed by nature, submit this foodstuff to a refining process which removes them, and then add them back to the refined product at an increased cost Yet this seems to be the thing that is being proposed If this is the object, why not follow the cheaper, more sensible, and nutritionally more desirable procedure of simply using the unrefined, or at most, slightly refined natural food" Sebrell (95) later reversed himself and actively supported the "enrichment" of white flour Taylor (88) suggested, "We should first seek retention of native vitamins"

In spite of this conservative and uncertain attitude toward fortification of foods, including the refinement of cereals, the Food and Nutrition Board of the National Research Council announced on January 29, 1941 its recommendation to enrich flour and bread Thiamine and nicotinic acid were essential additions to claim fortification Riboflavin was optional, mainly on account of its unavailability

The proponents of fortification of flour with thiamine and nicotinic acid freely admit the superiority of whole wheat bread, but insist that a start should be made with "enriched" white flour while the public would be educated to the advantages of whole wheat flour

The Council on Foods and Nutrition of the American Medical Association (89) accepted the "enriched" white flour because "thiamin is the component which makes whole wheat most significant in the diet at the present time" No attempt was made to justify this sweeping statement The Council pointed out the presence of factors other than thiamine in whole wheat not present in white flour, but this objection to white flour was conveniently sidestepped by calling these other factors "plus values" which the Council assumed without any supporting evidence, was furnished by the rest of the diet Yet they recognized that the "rest" of many poorer diets might not furnish these "plus values" Finally, they emphasized the high nutritional qualities of whole wheat and recommended undermilled flours Thus the Council on Food and Nutrition straddled the issue of whole wheat flour versus "enriched" white flour and failed to give the country the leadership so ably given to England by the British Medical Council Moreover, direct experimental evidence proved (70, 116) that the "plus values" were not present in the diet of large sections of our population The American Medical Association ignored the controversy over white and brown bread in England and the progress resulting from experimental investigations One year after the National Nutrition Conference in an editorial (113)

a whole chapter was devoted to "enriched" bread without any reference to events or progress in the intervening year Two years after the National Nutrition Conference, the Council on Foods (114) still emphasized thiamine and to a lesser extent riboflavin, in discussing "enriched" bread, no mention being made of accumulating literature on the inferiority of such enrichment compared to whole wheat bread

In May 1941 the National Nutrition Conference for Defense met at Washington to discuss nutritional problems created by the war emergency "Enriched" white flour was accepted without discussion The relative merits of "enriched" flour versus whole wheat flour as a national nutritional problem merited more consideration than it received Murlin (5) gave his consent "to this proposal of enrichment rather reluctantly," and pointedly remarked, "Six such factors are now available in chemically pure form, and without the achievements of the synthetic chemists working with the nutritionist we could not embark on the new program of vitamin enrichment of flour shall I say for better or for worse?" He also used the occasion to point out

1 Dogs fed the six synthetic vitamins remained deficient but the deficiency cleared up at once when the dogs were fed yeast, indicating essential, but as yet unknown factors in the vitamin B complex Digestion was notably improved by the addition of yeast

2 Pantothenic acid has been shown to have a favorable effect on digestion

3 Whole wheat bread would result in a great saving of food, in addition to other valuable factors

4 Studies made in Switzerland seemed to show that soldiers living on whole wheat bread maintained their fitness better than soldiers living on white bread

In place of critical discussion and experimental work there appeared a large volume of literature, monotonously similar, with the apparent object of proving to the American people that they will not eat whole wheat bread The unwillingness of people to eat whole wheat bread has been overemphasized Tobey (90) maintained that the average consumer is not keen about whole wheat bread as a steady diet, even if it is to his advantage to consume it "It has always been so and probably always will be, especially since our white breads are now virtually equivalent in nutritive properties to 100 per cent whole wheat The bakers and millers, by 'enriching' bread and flour, have succeeded in accomplishing what Mr Graham tried so vociferously, but failed so dismally to do, ' It is not true that "White breads are now virtually equivalent in nutritive properties to 100 per cent whole wheat" as shown by every nutritional experiment comparing them (52, 70, 83, 84 107, 116)

Taylor (91) in summarizing the various methods to correct the deficiencies of white flour referred to the return to whole wheat in the following manner, "Revival of old fashioned graham flour, despite its instability, dark color, bitter taste, high roughage and inadaptability to many commercial and household uses" This characterization of whole wheat flour is unfair Whole wheat flour does not have a bitter taste unless it is rancid Its instability is a commercial problem which has proved no obstacle in the past and certainly should

be less of one now As for the dark color of whole wheat bread, that will progressively become less of a hindrance towards its acceptance as people learn to appreciate that some vitamins are pigments and the dark color may become one of the commercial advantages of whole wheat bread Otherwise, Taylor (91) has given us an excellent discussion of the background of the nutritional problem involved in fortification under the title "Why enrichment of flour"

THE ARGUMENT OF PALATABILITY The unpalatability of whole wheat bread and the refusal of people to eat it, even if it is to their advantage, forms the foundation of the argument for fortifying white flour There is a fatalistic conviction on the part of the proponents of fortification that the general consumption of whole wheat bread is a thing of the past and as beyond recall as spilt milk (92, 93, 94, 95, 19)

Much has been made of the meager results attending the efforts of nutritionists to get people to eat whole wheat bread (93, 94, 95) It should be kept in mind that nutritionists have not until recently recommended whole wheat bread except in an academic way because they were under the influence of the doctrine of "protective foods" and whole wheat was not considered a "protective food" The term "protective food" was coined by McCollum and originally included only milk and green vegetables (96) The term was later extended to include fruits and eggs and in 1936 it was proposed to include meat also (96) Whole grain cereals were only then "nominated" for inclusion in the term "protective foods" The nutritionists taught "that in obtaining an adequate diet one must be sure to get the necessary vitamins and minerals first from other sources" (95), namely, the "protective foods," and then complete the diet with any food to suit the appetite such as white flour and potatoes Sebrell (95) pointed out that this resulted in a decreased consumption of flour, since the appetite was satisfied to such a large extent by "protective foods" It now appears that whole wheat bread because of its cheapness and abundance is one of our most important "protective foods," but the knowledge that whole wheat is a "protective food" is so recent as to have had no great effect upon its advocacy by nutritionists

Much is made of an attempt on the part of the Swiss Government to induce people to eat whole wheat and undermilled flours (97) In order to prevent a rise in the cost of living, the Federal Council of Switzerland put on the market a brown bread consisting of four-fifths wheat flour and one-fifth rye flour, the degree of extraction being fixed at 82 to 85 per cent This bread sold at a somewhat cheaper rate than ordinary bread Although no special propaganda was made for the bread, the consumption reached a level of 65 per cent of the total bread produced the first month, falling to 16 5 per cent nine months later, and finally after a year dropped to 11 per cent of the total bread consumption "The new cheap brown bread had, in fact, only replaced the other brown breads previously eaten and had not permanently replaced white bread to any appreciable extent"

This experience has been overemphasized as an argument showing that people will not eat whole wheat bread The report consisted of verbal information from Dr O Stiner, Federal Service of Health, Berne There is nothing in the

report to suggest whether it was the consumer or some difficulty in the nature of the flour or its treatment during milling or baking which was responsible for the decrease in consumption of the special bread. Furthermore an active educational campaign should always accompany a step of this kind.

While insisting that people won't eat whole wheat bread the proponents of "enrichment" of white flour ignore accumulating evidence that people are slowly but surely turning to whole wheat bread. They ignore in the first place that hundreds of millions of people are eating whole wheat bread and are liking it. The American people are no different as human beings from the European peoples.

In Aberdeen school canteens (98) experiments were conducted with the object of educating children to eat more wholesome food. While difficulties were experienced with some types of food, none was experienced with whole wheat bread, showing that it was palatable to these children.

In a study of actual foodstuffs consumed in various cities in Great Britain Cathcart and Murray (99) had this to say about St. Andrews, "The 'Bread' group provides two interesting items of information. The level of consumption of brown bread might almost be regarded as an index of social standing. Group I consisted of the highest income group. They all ate brown bread. As income progressively decreased, brown bread consumption decreased. Of the families in the lowest income group including the unemployed, only 1.3–8 per cent ate brown bread." Presumably the higher income groups consumed brown bread because they had been educated to its superior nutritive value and it must have been palatable or they would not have eaten it. Some white bread was eaten in all classes.

In a survey in Glasgow (99) in 1911 white bread was apparently universally eaten, whereas in 1933 about one-fourth of the families ate brown bread.

It appears therefore that there are other reasons besides palatability for the consumption of white bread or whole wheat bread. When people are convinced whole wheat bread is superior to white bread they find it palatable and when the two breads are compared, many find whole wheat bread more palatable than white bread. The unpalatability of whole wheat bread and the alleged refusal of people to eat it are myths and have no foundation in fact.

The "enrichment" of bread is frequently compared with the fortification of milk with vitamin D. That comparison does not stand scrutiny. Milk is a natural product and is practically a complete food, but it is low in vitamin D. Addition of vitamin D improves it beyond its nutritional value as a natural product, especially for children. Wheat on the other hand is first processed and converted from a food of high nutritive quality to a food of very low quality. To this nutritionally impoverished product thiamine, riboflavin and nicotinic acid are added and the product is called "enriched." Actually compared to whole wheat it is still nutritionally impoverished, and the term "enriched" bread is very misleading.

In their recommendations of daily allowances for specific nutrients (117) the Committee on Food and Nutrition fails to point out that these allowances are applicable only when natural foodstuffs comprise the diet. Then it can be

safely assumed that the unlisted members of the vitamin B complex as well as other factors are also furnished in adequate amounts. That assumption cannot be made when "enriched" bread forms a substantial part of the diet, since on paper such a diet will meet the recommended daily allowances of those specific nutrients mentioned, but will fail to supply a great many other nutrients not listed which may well be as important as those that are listed.

OPPOSITION TO ENRICHMENT. To this program for "enriched" bread, there has been no great opposition recorded in the literature, but apparently there has been a great deal of opposition which has not come out in the open as shown by the statement of Williams (94), "Yet this great reform is being sabotaged or damned with faint praise by half the nutritionists of the country on the ground that it would be still better if we could arrange breakfasts of ham and eggs, whole wheat buns and a glass of milk for everybody. Of course, it would be, but shall we wait for the millenium to take our first steps to mass repair our nutritional errors."

Luck (103) suggested that white bread be taxed as a luxury and the money thus obtained be used to subsidize whole wheat bread by establishing a price differential in its favor. This suggestion was coldly received or entirely ignored.

Carlson (104) contributed the following sound nutritional advice, "Nutritional safety lies in omnivorousness, in consuming, so far as possible, foods in their natural states, and, in the case of fruits and vegetables, eating some of them raw. Some of our malnutrition started with the processing, the refining, and the purification of such foods as the cereal grains, modern milling processes shunting the most valuable part of these natural foods into the mouths of chickens, cattle and hogs. The germ and the outer coats of the grains hold valuable proteins, minerals and vitamins. Human dietary safety on this front would seem to be: Go back to first principles, putting the whole grain into the flour and the bread. We can learn to like it. There is no more purity or virtue in white bread than in white winter butter. I think we could learn to prevent the oxidative rancidity of the whole grain. And until we have that problem licked, why not store the wheat and mill the flour as we need it.—In my judgment, the recent addition of a little of the vitamins and minerals now milled out of the grain, and singing paeans of dietary salvation over this 'enriched' flour and bread, is not a sound policy either for today or tomorrow." In fact, Carlson has been the only American scientist of note who has so far come out squarely for whole wheat flour as against "enriched" flour.

FURTHER EXPERIMENTAL WORK. *Cereals and calcium metabolism.* Evidence is rapidly accumulating to indicate there is a factor or factors in cereals which interfere with calcium metabolism. E. Mellanby (121) showed that on diets without vitamin D, rickets in puppies tended to be more severe on cereal than on non-cereal diets. He (122) later showed that under the conditions of his experiments, oats was the most rachitogenic of the cereals investigated and white flour the least, with but little difference between white and whole wheat flour. He demonstrated that vitamin D and calcium salts could counteract the rachitogenic activity of the cereals, but noted that inorganic phosphates were without

effect Since calcium salts could overcome the rachitogenic activity of cereals he found it necessary to explain why the cereals like oats with a high calcium content were more rachitogenic than white flour with a low calcium content This he did by assuming that cereals contained a "toxamin" which rendered calcium salts unavailable He found that boiling with 1 0 per cent hydrochloric acid destroyed this "toxamin," making the calcium of the cereal available for metabolic purposes This "toxamin" could also be rendered innocuous by "saturation" with calcium salts, after which dietary calcium would be available for metabolic purposes

May Mellanby (123) confirmed the destruction of the anticalcifying principle of cereals by boiling with 1 0 per cent hydrochloric acid She also studied the effect of germination on the anticalcifying principle of cereals and obtained irregular results She suggested that when loss of anticalcifying power did occur in cereals, it was due to enzymic changes

The possibility that the phytin of cereals might be the offending substance was entertained as a result of the investigations of Steenbock, Black and Thomas (124), Templin and Steenbock (125), and Bruce and Callow (126) These workers showed with rats that rachitogenic diets with a high calcium low phosphorus ratio could be corrected by the addition of inorganic phosphates Part of the rachitogenic effect of the diet was due to the unavailabity of the phosphorus in the phytin of the cereals Lowe and Steenbock (127) further showed that the availability of phytin phosphorus depended on the conditions of the experiment On low calcium low phosphorus diets, phytin could be hydrolysed in the gastrointestinal tract of the rat to a substantial though incomplete degree Calcium carbonate inhibited the hydrolysis of the phytin They established the absence of phytase, the phytin hydrolysing enzyme, in the intestinal mucosa of the rat and suggested that the phytase activity in the rat was probably due to the phytase of the flora of the gastrointestinal tract as well as the phytase of the ingested food

Harrison and Mellanby (128) insisted that the findings with rats on rachitogenic diets with a high calcium low phosphorus ratio had no bearing on his studies with puppies, since inorganic phosphates could not counteract the rachitogenic action of the cereal diet, but calcium salts could However, they investigated the possibility that phytin might interfere with calcium metabolism They found commercial phytin slightly antirachitic, but sodium phytate was definitely rachitogenic They could account for the rachitogenic action of cereals by their phytin content The phytin from cereals contained less calcium than commercial phytin The high calcium of commercial phytin could account for its slight antirachitic activity They (128) concluded "This view that the action of cereals is due to interference with calcium absorption accords with the observation that the effect can be prevented by feeding extra calcium, in other words, by saturating phytic acid and rendering it inactive" After saturating the phytic acid with calcium, further additions of calcium would presumably be available to the animal

While Harrison and Mellanby (128) had established that the phytin in cereals

was, at least in part, the offending rachitogenic substance, their contention that phytin rendered calcium unavailable by combining with it to form an insoluble calcium phytate was put into serious doubt when Krieger et al (129) showed that the calcium of calcium phytate was as readily absorbed as that of calcium carbonate when fed to rats on cereal-free diets low in calcium There remained to be explained the mechanism by which phytin interfered with absorption of calcium

McCance and Widdowson (130) made a thorough investigation with human beings of the absorption of calcium from diets in which either brown bread (made from flour of 92 per cent extraction) or white bread made up 40 to 50 per cent of the calories More calcium was absorbed from the white bread diet even though the calcium intake was less than that on the brown bread diet Fortifying the breads with calcium salts increased the absorption of calcium and prevented loss from the body if it had been taking place The carbonate was as effective as the phosphate Vitamin D did not improve the absorption of calcium in the human adult Sodium phytate when added to the white flour diet interfered with the absorption of calcium Yet, sodium phytate was split in the gastrointestinal tract since about one half of its phosphorus was absorbed (130) How phytin could be split to such a large extent and still interfere with calcium absorption was a puzzle It was explained on the assumption that calcium was absorbed in the upper part of the gastrointestinal tract, while phytin was split in the lower, and any unavailable calcium liberated in the splitting of the phytin was lost with the feces These investigators (130) showed that dephytinized bread lost part of its power to interfere with calcium metabolism, while phosphates were shown to account for the remainder of the anticalcifying action of brown bread The bulk of the feces had little to do with the absorption of calcium They attributed the poor absorption of calcium from brown bread to the specific action of the phytates and phosphates The dephytinization of bread deserves greater study The great variability among their subjects in their ability to absorb calcium was one of the outstanding findings of their study

On the basis of their studies (130) they recommended that flours be fortified with calcium as follows For each 100 grams of white, 85 per cent and 92 per cent flour, they recommended the addition of 65 mgm , 120 mgm and 200 mgm respectively of calcium When brown bread forms about 40 to 50 per cent of the caloric intake, a little less than a pint of milk would supply adequate calcium in the absence of other rich dietary sources of calcium

The problem of calcium metabolism is a complicated one The normal man secretes 4 to 10 liters of digestive juices containing 0 3 to 0 8 gram of calcium (131) Different types of food cause the secretion of different amounts of digestive juices Whole wheat bread will stimulate the secretion of a greater quantity of digestive juices than white bread (35) This calcium must be reabsorbed or lost to the body The absorption of calcium is influenced by phytates, phosphates, vitamin D, fat, citrates, oxalic acid, acid base balance and many other factors (131)

*Cereals and iron metabolism* Widdowson and McCance (132) cautioned

against laying too much stress upon brown bread as a food rich in iron This recommendation was based on some balance studies on white and brown bread with normal men and women More iron was retained on white bread than on brown, even though the latter bread contained more iron Positive balances were, however, obtained in all cases Data on balance studies, especially when positive balances are obtained, are hardly grounds for condemning the biological value of iron in whole wheat in the face of such overwhelming data in the rat indicating that the iron of whole wheat has a very high biological value as measured by hemoglobin formation (138, 107), a far more pertinent criterion than a small or large positive balance In this respect the work of Mitchell et al (107) is especially significant They showed with rats that a whole wheat diet induced a significantly higher hemoglobin production than an "enriched" white bread diet, even though the "enriched" white bread diet contained a little more iron Widdowson and McCance (132) cautioned against applying the results of experiments with rats to man because the rat secretes a phytase (133) and man presumably doesn't Therefore phytin would presumably interfere with iron metabolism in man but would do so to a lesser extent in the rat This criticism is, however, not valid There is no agreement on the ability of the rat to secrete a phytase since Lowe and Steenbock (127) could not find any phytase in the intestinal mucosa of the rat but attributed the phytase activity in the rat to the flora and the phytase of the food. Moreover, the human in this respect does not differ so markedly from the rat, since there must be a good deal of phytase in the human intestinal tract to account for the splitting of about half of the ingested phytin (130)

Studies on iron absorption seem difficult to interpret Widdowson and McCance (132) suggested that little iron is excreted in the gut except through bleeding and iron is poorly absorbed except when needed by the body These ideas have been amply confirmed (134) Patients bleeding from peptic ulcers absorbed 15 to 20 per cent of the iron administered whereas a normal medical student absorbed only 1.8 per cent Patients with hypochromic anemia absorbed a great deal more iron than normal people In general, it seems that the question of dietary iron has assumed an importance unjustified by the probable rôle it plays in the diet of the population (135)

*Feeding experiments with whole wheat and "enriched" bread* One of the most exhaustive and best planned investigations of the nutritional value of "enriched" white flour is that of Mitchell Hamilton and Shields (107) Three series of experiments were run The first series compared "enriched white flour, "enriched" white flour with 6 per cent skimmed milk solids, and white flour with milk solids The paired feeding method was used with equalization of food intake The rats were paired in trios The test showed that the flours containing the milk solids were biologically equal whether enriched or not, and superior to the "enriched" flour without the milk solids "Enriching" flour containing 6 per cent milk solids added nothing not already added by the milk.

In the second series of experiments, paired trios were again used comparing whole wheat flour, "enriched" white flour without milk solids and "enriched"

white flour with milk solids Again, the "enriched" white flour was inferior to the "enriched" white flour plus milk solids and also to whole wheat flour There was no significant difference in nutritive value between whole wheat flour and "enriched" white flour plus milk solids

In a third series of experiments, growth instead of food intake was equalized between the trios, thus comparing the nutritive value of the different flours in terms of the amount of food required by the rat to make the same growth In this series they compared whole wheat bread, whole wheat bread with milk solids, and white bread with milk solids The test showed that whole wheat bread was somewhat inferior nutritionally to white bread plus milk solids and whole wheat bread plus milk solids was superior to the other two breads It required 11 per cent less food as whole wheat bread plus 6 per cent milk solids than white bread plus milk solids for rats to grow at the same rate

Whole wheat bread gave somewhat better iron retention and hemoglobin formation than breads "enriched" with iron salts, with or without milk solids On the other hand, whole wheat impaired somewhat the utilization of calcium

Williams, Mason and Wilder (106) have evaluated the nutritive contribution of "enriched" white flour "with particular reference to satisfaction of the human requirements for thiamine and riboflavin " Seven subjects, all women, were used in this test and divided into 3 groups White flour, white flour enriched with thiamine, nicotinic acid and 6 per cent milk solids and whole wheat flour were compared The flour made up about 30 per cent of the calories of the diet, the rest consisting of foods commonly appearing on American tables The experiment was badly planned since neither the whole wheat flour nor the unfortified white flour contained milk solids, though it is a common practice of commercial bakers to add milk solids to white bread Properly planned, the experiment should have compared either white flour and "enriched" white flour or white flour with milk solids and "enriched" white flour and milk solids The omission of milk solids from whole wheat flour was perhaps justifiable since commercial whole wheat bread does not usually contain milk solids The addition of milk solids to commercial whole wheat bread should be encouraged

The subjects on the white flour diet developed symptoms of thiamine deficiency, but so did those on the "enriched" flour, though only in a mild form One subject on the whole wheat flour developed symptoms of thiamine deficiency and one was symptom free There were no definite symptoms of riboflavin deficiency in any of the subjects Their results could not be confirmed by Keys et al (118) who fed young men an equally low thiamine diet and subjected them to hard work No interference in carbohydrate metabolism nor any other symptoms of thiamine deficiency were noted

*The vitamin B-complex and hard labor* Johnson et al (108) investigated the effects of a diet, deficient in the vitamin B complex upon men doing manual labor They showed that men doing hard physical labor even for a few days need an adequate intake of the vitamin B complex Thiamine alone will not maintain the physical fitness of laborers, but whole dried brewer's yeast will This work has been confirmed in part by Barborka, Foltz and Ivy (109) who

showed that the vitamin B complex is necessary for hard physical labor. These workers used a yeast concentrate as their source of vitamin B complex.

The inability of thiamine to restore the physical fitness of men, deficient in the vitamin B complex, for doing hard physical work shows the danger of jumping to conclusions about the importance of thiamine in the human dietary. The rôle of thiamine in carbohydrate metabolism has led to the overemphasis in its importance in activities involving accelerated oxidations. To be sure, thiamine is necessary, but so are other factors and an adequate diet may be compared with a chain whose strength is determined by the weakest link. In the same way a diet becomes deficient if it is deficient in any single factor. To replace whole wheat whose nutritional quality is known, by an unknown quantity like "enriched" white flour is unwise. In this connection it should be pointed out that eight synthetic vitamins were inadequate as a source of the vitamin B complex for the monkey (119) and for the puppy (120). Should the intake of "protective foods" in our population be reduced below a safe level, the result may be nutritional disaster. This crisis is no time to play a guessing game with the nutritional welfare of 130,000,000 people at stake, especially when all evidence points to the nutritional superiority of whole wheat bread. The argument of palatability is dubious ground for such a course.

Composition of white flour, "enriched" white flour and whole wheat flour. While the nutritional superiority of whole wheat over white flour has been established for a long time by many feeding experiments, it is only recently that the reasons for this have been understood. As new factors of biological importance have been discovered, whole wheat and more especially the germ and bran were found to be good sources of these factors. In table 8 white flour, "enriched" white flour and whole wheat flour are compared with respect to all nutrients for which data could be found. The superiority of the whole wheat flour is impressive and explains the superiority found for whole wheat in feeding experiments. It should be noted that the table is incomplete, since other factors such as biotin, choline, inositol and folic acid are not listed for lack of data and they are, so far as known, also concentrated in the wheat bran and germ. Other less well known factors, and perhaps factors as yet undiscovered, are also likely to be present in these mill by-products.

It is difficult to see how in the face of this impressive evidence of the nutritional superiority of whole wheat bread, backed as it is by so many feeding tests, that the Committee on Food and Nutrition could recommend "enriched" white flour, at a time when "protective foods" of animal origin are becoming scarcer and are bound to become increasingly so under our war economy. They have recommended that we throw away our "staff of life" upon which we may have to lean very heavily and substitute in its place a crutch of known inferiority, of uncertain quality and untested value. It is altogether possible with our rapidly deteriorating food situation, if the war continues for any great length of time, *and especially in event of poor crops*, that we shall have to depend upon bread for a larger share of our food. Then the wide use of "enriched" bread might result in an impairment of the nutrition of large numbers of our people.

We do not know enough about food deficiencies in the population The clinical information is spotty and unreliable, since no reliable criteria exist to characterize these vague subclinical nutritional deficiencies The claims made for nutritional deficiencies in the population are based largely on dietary standards of various food factors and also nutrition surveys both of which are unreliable The dietary standards are little more than intelligent guesses at human requirements, and care has been taken to set them high enough to allow for margins of safety which is all to the good, but partial deficiencies of specific members of the vitamin B complex in the general population cannot at present be determined with any degree of certainty

TABLE 8

*Composition of white flour, "enriched" white flour and whole wheat flour*

| | WHITE FLOUR | "ENRICHED" WHITE FLOUR | WHOLE WHEAT FLOUR | REFERENCE |
|---|---|---|---|---|
| Thiamine, mgm per pound | 0 3 | 1 7 | 2 3 | (110) |
| Riboflavine, mgm per pound | 0 15 | 1 2 | 0 6 | (110) |
| Nicotinic acid, mgm per pound | 3 5 | 6 0 | 26 0 | (110) |
| Pyridoxine, mgm per pound | 1 0 | 1 0 | 2 0 | (101) |
| Pantothenic acid, mgm per 100 grams | 2 5 | 2 5 | 5 0 | (101) |
| Carotene (vitamin A), mgm per pound | nil | nil | 1 5 | (111) |
| α-Tocopherol (vitamin E), mgm per pound | nil | nil | 1 4 | (112) |
| Fat, per cent | 1 2 | 1 2 | 2 4 | (111) |
| Protein, per cent | 11 0* | 11 0* | 12 7† | (111) |
| Calcium, per cent | 0 02 | 0 02 | 0 045 | (111) |
| Phosphorus, per cent | 0 092 | 0 092 | 0 423 | (111) |
| Iron, mgm per pound | 3 0 | 6 0 | 20 0 | (111) |
| Manganese, grams per 750 calories | 0 1 | 0 1 | 6 7 | (86) |
| Potassium, per cent | 0 115 | 0 115 | 0 473 | (111) |
| Copper, grams per 750 calories | 0 40 | 0 40 | 1 6 | (86) |
| Ash, per cent | 0 37 | 0 37 | 1 70 | (111) |

* Low quality

† High quality

The proponents of enriched bread are on tenuous grounds when they claim

1 The major vitamin B complex defects of the American diet are thiamine and nicotinic acid

2 That these are the only valuable factors of the vitamin B complex supplied by whole wheat

3 That the rest of the diet contains an abundance of the other vitamin B complex factors (plus factors) of whole wheat, thus justifying their removal in the milling process

The plain truth of the matter is that we do not know It would be advantageous to use whole wheat bread for the following reasons

1 The vitamin B complex with the exception of riboflavin would automatically be taken care of Losses in cooking would not have such a serious effect on the diet

2 Unknown factors would be supplied giving greater confidence to the diet, since some of them may not be adequately furnished by the rest of the diet

3 Proteins of high quality and minerals would be furnished

4. Milk would supplement the wheat by making good the riboflavin deficiency, improve the quality of protein and add enough calcium to make it adequate. Milk production is being encouraged at the expense of meat production.

5 With milk and whole wheat bread the diet would practically be adequate and addition of small amounts of eggs, fish, fruits and vegetables would round out the diet and make it nutritionally excellent

6 It is always far easier to build an adequate diet around whole wheat flour than white flour

7 As foodstuffs such as meat, eggs, milk, fruits and vegetables become scarce as a result of the war, the whole wheat, if used, will become the mainstay of our diet and if properly combined with what protective foods will be available, the nutritional level of the population will remain high If white flour is used, even if "enriched," it is difficult to see how deterioration of the national diet can be avoided That would indeed be a matter of grave concern

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# THE CHANGES IN THE FETAL CIRCULATION AT BIRTH

DONALD H BARRON

*The Laboratory of Physiology Yale University School of Medicine*

The pattern of the fetal circulation and the changes it undergoes when breathing begins at birth have long been topics of interest and speculation, but seldom subjects of direct observation As a result the greater part of our knowledge about them was until recently, inferred from observations made post mortem upon fetuses at verying stages during gestation and upon the new born Within the past ten years, however, a great deal of information has been accumulated by direct methods in studies carried out in, and inspired from, the laboratories of Sir Joseph Barcroft at Cambridge and A E Barclay at Oxford

As is often the case the advances in our knowledge have resulted from the application of new techniques to the age old problems Barcroft has studied intensively the oxygen content of the fetal blood to discover the pattern of the circulation Barclay has brought to a high degree of perfection methods for making x ray cinematographs These methods have enabled him and his collaborators to record pictorially the movement of radiopaque substances such as thorotrast, uroselectan B, and lipiodol, after their introduction into the blood stream, and so the movement of the blood The records are of two types direct and indirect. The direct records are made by exposing five inch segments of a continuous film five inches wide and many feet in length Such exposures have been successfully made at a rate of three or four per second Indirect records are made by photographing with a sixteen millimeter cine camera, at normal speed, the images made by the x-rays on a flourescent screen

The application of these new techniques has clarified our views, but even they do not permit the study of the circulatory changes *in utero* and at normal birth To apply them the fetus must be delivered by caesarian section Under the most favorable conditions the uterus after delivery begins to contract and impair the circulation through the fetal and maternal sides of the placenta Until someone devises methods for study of the fetal circulation *in utero* we shall be obliged to deduce its character from fetuses under such conditions Despite this limitation the available data do serve to broaden our view about the nature of the vascular system in prenatal development and at birth To this end they are collected here

*The pattern of the fetal circulation immediately after delivery by caesarian section, placental circulation intact* Radiopaque substances introduced into the umbilical veins of near term fetal lambs (27) are carried to the posterior vena cava along either one of two routes One leads from the umbilical recess—the proximal portion of the vein formed by the union of the two umbilicals—through the liver capillaries From these capillaries the blood is collected by two large hepatic veins and a varying number of small vessels all of which empty into the posterior cava The second route leads from the umbilical recess through the ductus venosus—thus avoiding the liver capillaries—into the vena cava

There has been speculation from time to time as to the portion of the return from the umbilical veins that follows each of these routes. The films of Franklin, Barclay and Prichard (27) demonstrate that in the sheep the greater part goes through the liver capillaries, the smaller through the ductus venosus. This evidence is in accord with the older anatomical studies which indicated that the ductus venosus in the human fetus at term had a diameter ¼ of that of the umbilical vein (54). The portion of the blood traversing the ductus may—under circumstances not well understood—be diverted into the liver capillaries.

The x-ray records of fetal lambs reveal that the flow through the ductus may cease abruptly only to begin again after intervals of varying length. The cessation and renewal of the flow through the ductus venosus resembles in some respects the arrest of the blood flow through the ductus arteriosus at birth (see below). It appears to be brought about, as Barclay and his colleagues suggested (8), by the contraction of a horseshoe shaped sphincter muscle in the wall of the umbilical recess, just at the point where the ductus springs from it (20). This muscle is supplied, in the sheep, with motor type endings by a nerve formed by union of branches of the two vagi. These nerve fibers do not appear to end on ganglion cells in or near the umbilical recess but directly in its musculature. Hence they are probably not parasympathetic preganglionic but post ganglionic sympathetic fibers. This assumption is supported by the observation of Franklin (personal communication) that stimulation of the vagi in the neck region of fetal lambs was not followed by constriction of the sphincter in a single experiment. Further observations will be necessary before any final statement can be made.

Studies of the activity of this sphincter as revealed by x-ray cine records have been made only in fetuses near term. It may be that the sphincter is active only in the closure of the ductus at birth. However, the peripheral mechanism is laid down and anatomically complete by the fortieth day of gestation (20) so that it may play a rôle in regulating blood flow through the liver in normal uterine life. If active, it might be expected, by constricting, to raise the blood pressure in the umbilical veins and to lower it in the cava. As will be pointed out below, lowering the pressure in the posterior cava might have important consequences at the heart.

To return to the course of the blood leaving the liver. In the posterior vena cava the blood bearing the contrast material is added to the return from the caudal half of the body. Upon its approach to the heart, as indicated by the x-ray records, this stream divides (5). The deeper shadow indicating the larger stream of blood enters the left heart via the foramen ovale, the smaller stream, casting a shadow less dense enters the right atrium.

In the left heart the stream from the posterior cava is added to the return from the lung capillaries and projected through the aortic arch into the head, the upper extremities and the dorsal aorta. The stream into the right heart is added to the entire return from the upper circulation arriving via the anterior vena cava, for the radiographic studies indicate that none of the anterior caval flow enters the left heart directly. The contents of the right heart are projected—as indicated

by the shadows cast by contrast materials borne in them—along the pulmonary aorta, to the lung capillaries and through the ductus arteriosus into the dorsal aorta The blood in the dorsal aorta represents contributions from both ventricles This pattern of the fetal circulation appears to be substantially the same through the last third of the gestation of the fetal lamb It can be represented diagrammatically as in figure 1

Similar results with regard to the course of the two caval streams into and through the heart have been obtained in kitten and guinea pig fetuses by Windle and Becker (51) Their methods were simple and direct In these fetal types,

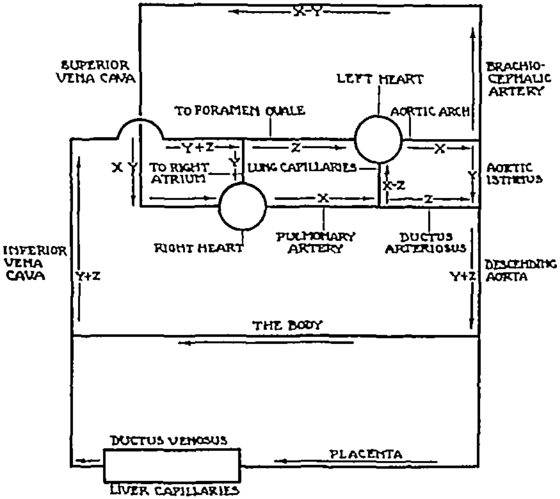


Fig 1 A scheme representing the basic plan of the fetal circulation The arrows indicate the direction of blood flow The blood flow per minute in each of the major channels is expressed algebraically For details see text

if the chest plate is removed, the atrial walls are thin enough to permit the detection of small differences in the color of their contents by inspection India ink in small quantities was introduced into either the umbilical or jugular veins Ink introduced into the umbilical vein passed for the most part directly into left atrium as indicated by its darkening a small quantity of ink entered the right atrium Ink injected into jugular vein did not darken the left atrium on reaching the heart, but passed through the right atrium and ventricle to the ductus arteriosus and the lungs

Some of the consequences of this pattern of circulation have been revealed by a study of the oxygen content of the blood in the umbilical vein and arteries,

and in the carotid (30, 13). In the fetal lamb the amount of oxygen in the carotid blood is intermediate between that in the dorsal aorta—as represented by the umbilical artery—and that in the umbilical vein. Until a few days before birth it is always nearer the value for the umbilical artery than that for the vein, but in the last days of gestation the oxygen content of the blood in the carotid tends to drop to a value nearer that of the umbilical artery. Substantially the same results were obtained by Huggett (30) on goat fetuses, though his series is not so complete nor does it include observation on fetuses in the very last days of gestation.

These three lines of evidence indicate that the blood returning from the placenta rich in oxygen is distributed in the main to the upper circulation, which includes the heart itself, the upper extremities, the central nervous system and a part of the chest wall. But the significance of this distribution remains to be discovered.

*The anatomical basis of the pattern of the fetal circulation.* The establishment of the pattern of the circulation through the fetal heart raises the question: How is this selective distribution of the two caval streams accomplished? Apparently the failure of any one in the past to demonstrate to general satisfaction any mechanism responsible for a selective distribution of the two streams in the heart has proven the greatest obstacle to a widespread acceptance of the Sabatier (47) theory. Sabatier believed that all of the blood reaching the heart from the inferior vena cava passed directly into the left atrium via the foramen ovale without entering the right atrium. From the left atrium it was distributed to the head and upper extremities. The blood returning to the heart from the superior vena cava, he believed, passed through the ductus arteriosus into the descending aorta thence to the lower parts of the body and the placenta. None—according to the hypothesis—passed through the lungs, none through the isthmus of the aorta.

The opposing view, erroneously credited to Galen and Harvey (see Franklin, 26) assumed that the two caval streams mixed in the right atrium and the left atrium was supposed to be supplied via the foramen ovale from this mixture. Both ventricles pumped blood of the same composition into their respective beds. The circulation through the lungs was assumed to be minimal. This view has been supported, to be sure, by anatomical and physiological studies (43, 44, 32, 33, 39, 40) but its popularity appears to have stemmed in a great measure from its inherent probability. This inherent probability, of course, rests on the assumption that the two cavae end in the right atrium and only there, and further that the foramen ovale is the only direct communication between the two atria. These assumptions—a part of current teaching of the embryology of the heart—have had their contestants along the years, chief among them Wolff (53), Kilian (36), Rudinger (46), Zeigenspeck (54) and recently Amoroso *et al.* (2).

Wolff was apparently the first to advance, on the basis of anatomical studies made upon human fetuses, the view that the atria in the fetal heart are not in direct communication with one another, but are joined through the channel of the inferior vena cava which opens separately into each chamber. Wolff's con-

tention was supported later by the anatomical studies of Kilian (36) and of Zeigenspeck (54) In his descriptions of the termination of the cavae in Preyer's book, *Die Physiologie des Embryos* (45), and in his later paper (54) Zeigenspeck provided illustrations and diagrams that would appear to establish Wolff's view as a fact, but these were either ignored or did not come to general attention

Lately the subject has been restudied by Amoroso *et al* (2), who pointed out that in the light of recent embryological studies a part of Wolff's inferior vena cava would not be so described now, for it arises from cardiac tissue However any difficulties that might arise from the use of anatomical terms with fixed implications may be avoided by the use of their term "posterior caval channel" for designating the structure as it appears in the fetal lamb Under this term Amoroso and his colleagues would include not only the posterior cava itself,

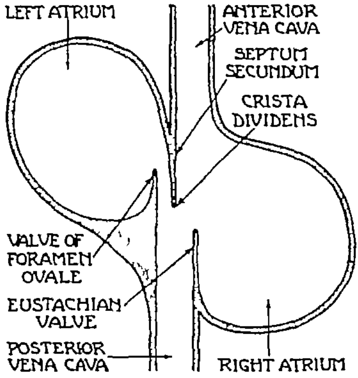


Fig 2 A diagram illustrating the relations of the termination of the posterior caval channel and the interatrial septum in a mid term fetal lamb

but also the intracardiac continuation of the channel up to the level at which the posterior caval blood discharges into the atrial cavities In a study which included a variety of forms—ungulates, carnivores and primates—but gave special attention to the lamb, Amoroso *et al* found that the division of the posterior caval channel into the right and left branches occurs on the free edge of what embryologists in this country would term the septum secundum or isthmus atriorum They purpose the term "Crista dividens" to designate this edge—a term surely more indicative of its functional rôle but one which gives little hint of its embryological origin In the fetal lamb at term (see fig 2) the left boundary of the left fork of the posterior caval channel is the valve of the foramen ovale or septum primum, the right boundary of the right fork of the channel opening into that atrium is formed by the Eustachian valve or the right valve of the

sinus venosus The left fork of the post caval channel leads, therefore, directly into the left atrium, the right into the right atrium According to Amoroso *et al* (2) this general plan is found in the other forms they studied

This view of the configuration of the termination of the inferior vena cava and the position of the foramen ovale has been repeatedly opposed (21, 44), especially by Born who objected that the relationships of the parts could not be established on fresh specimens in which they could be pushed and pulled about, an objection that seems to have been regarded as valid by the vast majority of embryologists for Wolff's interpretation does not appear to have been generally accepted

Further anatomical studies will doubtless be needed before Wolff's views become a part of current teaching, but the fact remains that they do account for the results which have been obtained from the studies on the course of the posterior caval blood through the heart These studies in turn are a confirmation of his scheme of the fetal circulation, for Wolff expressed the opinion about one hundred and sixty years ago that the posterior caval blood was poured into both atria—one-third of it into the right, two-thirds into the left After one hundred and sixty years these views may be regarded as substantially correct for the final stages of development

One further anatomical point merits attention, how is the anterior caval blood prevented in the fetus from entering the posterior caval channel and so entering the left heart? This separation appears to be accomplished by a tubercle—the tubercle of Lower—interposed between the central ends of the two cavae According to the description of it given by Franklin, Barclay and Prichard (27), this tubercle in the fetal sheep forms a part of the dorso-anterior wall of the posterior cava and the postero-dorsal wall of the anterior Its rôle in the separation of the caval streams they have deduced by a comparison of radiographs of the roots of the great veins with dissections of them

The structure of the heart and the roots of the great veins appears, therefore, to be compatible with, and in all probability responsible for the course of the blood into the two ventricles If the rôle of these structures in the functional separation of streams to the right and left hearts needs further emphasis it has been provided in a striking manner by Whitehead's (50) neoprene model—a model based upon a reconstruction from serial sections, of the right atrium, the caval terminations, the foramen ovale and the right atrioventricular opening of a fetal kitten killed *in utero* When perfused via the "inferior vena cava" with red colored water at 75 mm Hg and at the same time through the "superior cava" at 67 mm Hg with blue, the fluid which emerged from the "foramen ovale" contained 92 per cent red from the "inferior cava" and 8 per cent blue from the "superior" The fluid leaving the "atrioventricular orifice" was 87 per cent blue from the "superior cava" and 13 per cent red from the "inferior" The position of the model in space had no effect upon the results However, when the pressures were lowered and equal the two "caval" streams mixed completely before emerging at the "foramen ovale" and the "atrioventricular opening"

*The rôle of dynamic factors in the maintenance of the fetal circulation pattern* There are suggestions, though no direct evidence, that in the living heart as in

Whitehead's model, the pressure in the posterior cava must exceed that in the anterior if the normal distribution of the streams to the atria is to take place For example, Windle and Becker (51) noticed that the caval streams underwent rather thorough mixing in the atria of kitten and guinea pig fetuses when the heart slowed and the blood pressure fell A fall in the blood pressure of the fetuses they were studying may have been the underlying cause for the mixing of the caval streams in the heart as observed by Pohlman (44) in pigs and by Kellog (32) in dogs—an observation that led both investigators to assume that the two caval streams normally mixed in the right atrium Their results really bear witness to the fact that the distribution of the caval streams observed by Barclay *et al* (4, 5) and Windle and Becker (51) depends upon dynamic factors in addition to those of configuration

And these dynamic factors may, in some fetal forms at least, be altering a good deal from moment to moment during the last day of gestation During that period, as is well known, the uterine contractions that earlier occur so occasionally increase in frequency Each uterine contraction alters the fetal blood pressure by arresting circulation through the vessels of the placenta

In the fetus a transient rise is followed by a well defined fall which lasts throughout the contraction During the fall in pressure the fetal heart is slowed (23) This fall in pressure, other things being equal, might be expected to be accompanied by mixing of the caval streams in the heart Similarly the narrowing of the ductus venosus and a fall in the posterior caval pressure might be accompanied by mixing of the caval streams *In utero* the occurrence of these events certainly could not be regarded as abnormal Perhaps we would not be far wrong if we assume that the distribution of the caval streams, as pictured by Barclay *et al* (4, 5) and by Windle and Becker (51), represents the initial pattern from which the transition to the adult type begins—the changes in the dynamic features as in the nature of rehearsals for the first act of the drama of birth

*Variations of the circulation within the fetal pattern* There is good evidence from the studies of Barclay *et al* (4, 5) that the pattern of the circulation remains unaltered during the last third of gestation But within this pattern there are possibilities for wide variation in the proportions of the blood that perfuse the individual channels Data indicating the extent of these variations are meager indeed, but certain tentative conclusions may be drawn from them

Four facts appear to be established about the fetal circulation 1 That the posterior caval stream divides, a part going to each atrium 2 That the anterior caval stream enters only the right 3 The capacities of the two ventricles are equal (44) as are—4—the pressures in them (20) Taken together the last two statements indicate that the ventricular outputs are equivalent These facts may be related to the scheme of the fetal circulation as illustrated in figure 1 In this scheme the output of each ventricle may be called $x$ The output of the left is divided between the upper circulation and the aortic isthmus The fraction perfusing the isthmus may be designated $y$, then the quantity passing through the upper circulation will be $x - y$ This quantity $x - y$ is returned to the right heart To provide the output $x$, the quantity $y$ must be furnished by the stream

entering the right heart from the inferior vena cava The minute volume through the aortic isthmus appears therefore to be equivalent to that of the stream from the inferior cava into the right heart

The output of the right ventricle $x$ is divided between the lung circuit and ductus arteriosus The minute volume through the ductus arteriosus may be designated $z$, that of the lung circuit then becomes $x - z$ The quantity $z$ from the ductus is added to the $y$ from the aortic isthmus and $y + z$ then represents the flow through the dorsal aorta to the body and placenta which returns through the posterior cava Since the quantity $y$ is diverted to the right heart, the quantity $z$ must reach the left heart via the foramen ovale to be added to the stream represented by $x - z$ from the lungs Upon this reasoning the minute volume through the ductus arteriosus appears to be equivalent to that through the foramen ovale According to this scheme if $z$—the flow through the ductus arteriosus—equaled the output of the right ventricle, $y$ would be zero The two ventricles would then be operating as pumps in parallel and the pattern of the circulation would be similar to that postulated by Sabatier (47) On the other hand, as the value of $z$ falls the proportion of the right ventricular output perfusing the lungs increases, when $z$ equals zero, then all of the output of the right heart will perfuse the lungs and the flow through the foramen ovale and the ductus arteriosus will cease The two ventricles would then be arranged to pump in series One further generalization can be made about this scheme Any increase in the minute volume of blood traversing the lung capillaries will be accompanied by a corresponding decrease in the minute volume through the lower circulation Oddly enough the minute volume through the upper circulation remains unchanged

*Observations upon the relation between the quantities $y$ and $z$* One of the conclusions arrived at above was that the division at the heart of the posterior caval stream into two parts was primarily a result of the configuration of the termination of the posterior caval channel Professor Patten (private communication) has informed me that his studies of the relations of the posterior caval channel and the foramen ovale indicate that there is no pattern standard in all mammals and that the relations alter during development of an individual The differences are largely the result of variations in the position of the septum secundum with reference to the termination of the posterior caval channel The comments of Professor Patten recall the observations of Wolff (53) and Zeigenspeck (54) Wolff concluded from a study of the human fetal heart that the septum secundum was placed so far to the right with respect to the midline of the inferior caval channel that, until the third month of fetal life, almost all of the inferior caval blood was poured into the left atrium As development advanced the valve of the foramen ovale grew and the septum secundum shifted relatively toward the left Wolff estimated from the position of the septum secundum that near full term one-third of the inferior caval blood went into the right atrium

The recent study of Keen (31) amply confirms Wolff's contention about the shift of the septum secundum and his excellent illustrations should be studied by

everyone interested in the subject In the heart of the human fetus of 28 weeks illustrated by Keen the inferior caval channel opens almost directly into the left atrium, i e, completely to the left of the septum secundum, whereas in the human heart at birth the channel opens very much farther to the right The septum secundum is placed very near the left wall of the inferior caval channel but still separated from it

According to Zeigenspeck's (54) estimate—again based upon the position of the septum secundum in relation to the opening of the posterior caval channel in human fetuses near term—one half of the blood goes into the right atrium This estimate has support from observations of Patten and Toulmin (42) who have calculated the cross sectional areas of the inferior vena cava and of the functional orifices of the foramen ovale in twenty stillborn babes The cross sectional area of the cava they found to be 69 7 sq mm, the orifice of the foramen ovale 32.2 sq mm Inasmuch as the pressures at these two points are the same, the flow to the left side of the heart at term would appear to be just about half of the inferior caval return, $y$ then, on the basis of these studies would appear to be small relative to $z$ early in gestation but to increase gradually until finally at term it is equal to $z$ or nearly so

These estimates of $y$ furnish, when applied to the scheme in figure 1, an estimate of the quantity of blood traversing the isthmus of the aorta for these quantities appear to be equivalent. At term the ductus arteriosus and the isthmus must both be contributing about the same quantities of blood to the descending aorta This supposition has further support from Patten and Toulmin's (42) observation that the cross sectional areas of the isthmus and of the ductus are nearly equivalent (actually 14 4 and 15.2 sq mm respectively) The pressures in the vessels are equal, hence the flow is in all probability the same in each

There are other ways of estimating the proportion of the posterior caval flow following each route into the heart and it is to be hoped that they will be applied to the problem Since lipiodol injected into the umbilical vein forms droplets that are carried in the blood, it should be possible radiographically to record, as Barclay and his colleagues have done, the results of such an injection and then to determine the number of droplets that followed each route through the heart Other things being equal the drops should be in proportion to the quantity of blood following each path Until some such technique is employed, probably no more definite conclusions can be drawn than the estimates arrived at above

*Observations on the relationship between the quantities $z$ and $x$* The estimations made above which place $y$ at the end of term equal or nearly so to $z$, imply that the quantiy $x - z$ must be nearly the equivalent of $y$ and of $z$ In other words, they imply that at the end of gestation a very large proportion of the output of the right heart is perfusing the lungs, a contention advanced by Patten (39) on anatomical grounds

This is certainly not true of sheep and goat fetuses during the first two-thirds of gestation The studies of Huggett (30) on goats and Barcroft and his collaborators on sheep (10, 13) have shown quite conclusively that the amount of oxygen in the blood of the carotid is intermediate between the oxygen in the bloods

of the umbilical vein and artery (see fig 3) In the sheep fetus until about 40 days before the end of term the percentage saturation of the carotid blood so nearly approaches that of the umbilical vein and differs so markedly from the blood of the umbilical artery, that after making due allowance for the contamination of the umbilical vein blood by the contents of the posterior vena cava, there can be little, if any, further reduction in the left atrium The inference is of course that $x - z$ or the flow through the lungs is very small indeed Therefore, $z$ must be very nearly equal to $x$ during this period and Barcroft's estimate (9) that the ductus arteriosus conveys about half of the cardiac output to the lower circulation cannot be far wrong

During the last 40 days of gestation (13) the oxygen content of the carotid blood tends to approach that in the umbilical artery and is very near it in the last ten days The oxygenated post-caval blood is apparently diluted to an ever increasing degree, during this period, by reduced blood This of course does not prove that the mixing or dilution occurs in the left atrium, merely that the fall in the oxygen content of the carotid blood would not be incompatible with an increased return through the lungs The mixing might occur as a result of an increased return through the body circulation into the posterior vena cava—a view favored by Barcroft *et al* (13)—or as a result of regurgitation of the right atrial contents into the left via the posterior caval channel For the latter two possibilities there is not a great deal of supporting evidence

The view that the mixing does take place as a result of an increased lung flow is supported by the radiological studies of Barclay *et al* (4, 5), for a considerable portion of the contrast material introduced into the external jugular vein finds its way into the lungs from the pulmonary aorta (The proportion could be determined for any stage by the use of lipiodal drops as indicated above ) From these studies, too, comes the only indication we have of the circulation time through the lungs of the fetus (8) As judged from the time required for radiopaque substances to be carried through the lung capillaries from the pulmonary arteries to the left atrium, in quantities sufficient to produce a shadow on the film, the average circulation time is remarkably short, *i e*, 2 7 seconds, and surprisingly enough this figure does not appear to alter significantly during the last forty days of gestation The blood in the lungs of the fetal sheep is therefore renewed about twenty times a minute, a surprisingly high rate

The volume of the blood in the lungs of the fetal sheep during development has not been determined, hence we have no real idea of the quantity of blood involved However, the vascular bed of the lung does not make any spectacular growth in the last ten days of gestation, during which, if born, the fetus will survive, so its size and the amount of blood in it during these days must be about equivalent to that in the newborn lamb At least this appears to be the case in the fetal types in which the blood volume of the lung has been estimated Abel and Windle (1) have determined the total iron content and found equivalent amounts in the lungs of full term kittens before birth and after breathing The inference is that the amount of hemaglobin and hence the amount of blood was the same in both circumstances In the human fetus at term the lungs have been

found to contain 5 per cent of the total blood volume (37), the normal amount in the adult lung is usually given as between 5 and 7 per cent If any similar proportion of the total blood volume is present in the lung of the fetal lamb, during the last ten days of pregnancy, the amount circulating through the lungs must be very large indeed for the estimated blood volume of the fetus near term is between 300 and 350 cc

Another bit of evidence, also indirect, indicating the quantity of blood perfusing the lungs of the fetal lamb near term, comes from the observations of Barcroft (9) on the amount of blood (z) carried by the ductus arteriosus The ductus from a 144 day fetus, perfused with cold blood at a pressure of 103 mm. Hg at the pulmonary and 58 at the aortic end, carried 88 cc of blood per minute This quantity may be less than the ductus would carry in situ, but it is certainly of the same order and it is one which is more than a third, probably a great deal nearer a half, of the output of the right ventricle per minute The output of the fetal lamb heart is probably very near the same as that of the fetal goat, *i e*, 0 12 to 0 18 cc per gram of fetus per minute (19) or about 525 cc per minute for a 3500 gram fetus or about 262 cc per minute for the right ventricle Zeigenspeck (54), one may recall, estimated that at the end of term half of the output of the right heart went through the lungs

Thus arranged, these data indicate that early in gestation nearly all of the post-caval blood enters the left atrium, a very small part is diverted into the right At this time very little blood is passing along the aortic isthmus and the ductus arteriosus is carrying very nearly the entire output of the right side of the heart Under these circumstances there is a wide difference between the oxygen content of the carotid blood and that in the dorsal aorta, the "crossing" of the streams is nearly complete With advancing development the septum secundum gradually shifts to the right relative to the midpoint of the post-caval channel As a result of this shift and accompanying it there is a gradual increase in the proportion of the post-caval blood entering the right atrium Hand in hand with this increase the proportion of the left ventricular output traversing the aortic isthmus increases, the flow through the ductus arteriosus decreases and that through the lungs increases These changes bring about a gradual increase in the oxygen content of the blood in the right heart and consequently that of the blood traversing the ductus arteriosus and the lung capillaries As the contribution from the posterior cava via the foramen ovale decreases and the lung circulation opens up, the oxygen content of the blood in the left heart and the carotid gradually fall Contrariwise the oxygen content of the blood in the dorsal aorta increases as the contribution from the aortic isthmus grows and that from the ductus arteriosus declines As a result the oxygen content of the carotid blood and that of the dorsal aorta—so different in early development—approach each other as development advances and are equivalent or nearly so at the end of gestation That this is what does happen is indicated by the studies of the blood gases (13, 17) see figure 3

*The changes in the pattern of the vascular system at birth by caesarian section* The uterine contractions that force the fetus into the external environment also serve to initiate the series of interdependent steps that result in the produc-

of the adult circulation pattern They serve to impede the placental circulation and to transfer a large part of the placental blood—25 to 30 cc per kilogram of fetal weight in the case of the lamb (14) and about the same proportion in the human infant (25)—to the fetus This transfer and the decline in the placental circulation appears to result in the asphyxiation of the fetus and asphyxiation in turn in the initiation of respiration *In utero* the respiratory center appears to be stimulated by $CO_2$ excess (52), $O_2$ want (10) and through the activity of somatic sensory nerves (30, 11, 12) All of these influences must be brought to bear upon the medullary center to an ever increasing degree as the uterine contractions increase in frequency and force Their relative importance and rôle in initiation of respiration is beyond the scope of this review Important for the

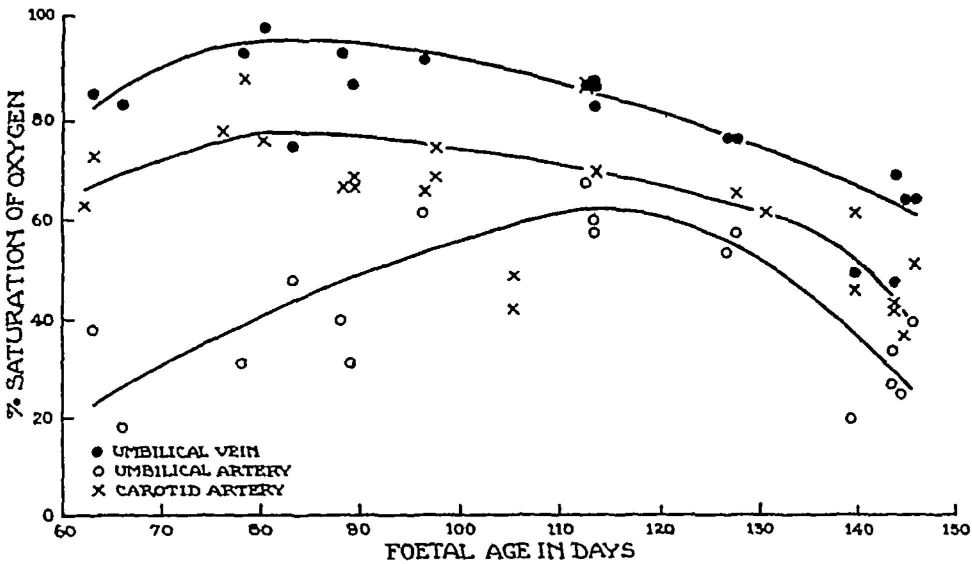


Fig 3 A graph based upon Barcroft's data illustrating the variations in the oxygen content in the umbilical vessels and in the carotid artery of the fetal lamb as development advances

present purpose is that they succeed, for the initiation of respiration is a *sine qua non* for the changes in the vascular system Lamb fetuses whose medullae are destroyed *in utero*, or whose cords are sectioned anywhere between C5 and the calamus scriptorius, always die with the pattern of the circulation unaltered from the fetal type (Barron, unpublished) However, respiratory movements alone do not suffice to produce the change Lamb fetuses killed *in utero*, after executing respiratory movements, by injecting formalin into the heart always have the fetal pattern of circulation (see Barcroft, 10) The respiratory movements must succeed in ventilating the lungs

The effects of breathing on the circulation of the fetus have been studied in some detail by Hamilton *et al* (29) in dogs and rabbits and by Barcroft in sheep There are minor differences but the main features appear to be shared by all

three fetal types In dog and rabbit fetuses, before breathing, the systolic pressure is about equal in both ventricles as is the diastolic pressure in the two auricles During early breathing the diastolic pressure falls in both ventricles and the systolic pressure of the left falls the same distance The systolic pressure of the right ventricle, however, drops three or four times as far The pressures rise and fall during the heart cycle but at the lower level The heart rate is increased The fall of the diastolic pressure in the two ventricles and the systolic of the left were attributed by Hamilton *et al* (29) to the decrease in the intra thoracic pressure which results from the expansion of the thorax The further fall in the right systolic was attributed to a change in the peripheral resistance in the lesser circulation

In the sheep fetus there is no record of the pressure in the right ventricle but the systemic blood pressure rises 30 or more mm Hg (10) with breathing, and the circulation time in the pulmonary circuit falls from 2 7 to 1 4 seconds (8) indicating a decrease in the pulmonary resistance—a decrease that would be compatible with a fall in pressure in the pulmonary artery The difference in pressure between the pulmonary circuit and the systemic, that is established as a result of breathing, led Hamilton *et al* (29) to postulate an early closure of the ductus arteriosus For were it to remain patent, blood would flow along the ductus arteriosus from the aorta to the pulmonary artery which is the opposite direction to that of the fetal stream

*The functional closure of the ductus arteriosus* This postulate has been substantiated for, as indicated by x ray cine records (4, 5), the ductus arteriosus can and does close in the lamb within four minutes after the placental circulation is arrested and the respiratory passages are opened by the removal of a bag placed on the nose of the fetus before it was taken from the amniotic fluid of the uterus In these four minutes air had entered the lungs and outlined the bronchial tree in the radiograph. The closure may not always be so abrupt and complete The ductus may close, reopen and oscillate between complete and nearly complete closure for fifteen or more minutes after ventilation of the lungs is established The reopening of the ductus appears to occur as the animal deteriorates—as the respiratory movements grow less successful in oxygenating the blood Simultaneously the circulation time through the lungs increases In fact there is a suggestion that the degree to which the ductus is closed is directly related to the pulmonary circulation time (8) Both of these happenings are probably correlated with the decrease in the peripheral resistance in the lung and hence with the fall in the systolic pressure in the right ventricle

This prompt functional closure of the ductus arteriosus associated with the initiation of respiration requires a mechanism that can obliterate the ductus suddenly, and if need be to reopen it, in short, the action of a sphincter The capacity of the ductus to contract and relax suddenly like a sphincter has been observed directly in full term guinea pig fetuses (16 34) With the chest opened, faradic currents applied directly cause it to narrow abruptly as it does if manipulated (16) In the light of these observations and the radiological evidence the view that the ductus is a structure whose functional rôle diminishes

over a relatively long period of time coincident with its anatomical degeneration and obliteration is no longer tenable The opinion of Gérard (28) that the obliteration of the ductus embraces two stages—1, the physiological occlusion, which occurs very shortly after birth, leaving the ductus patent in all its length but preventing the passage of blood, and 2, the anatomical obliteration—now appears fully justified

The ductus functions as a sphincter and all recent descriptions of its histology agree that the walls contain structures associated with sphincters (49, 22, 34) According to Von Hayek the tunica media is composed almost entirely of muscle fibers loosely arranged and coiled about the lumen like a long spring There is an outer layer in which the fibers forming the coil are very nearly circular The fibers of the inner layer tend to be more longitudinally disposed These fiber bands are arranged to coil in opposite directions so they cross each other at a near right angle The elastic tissue layer is very poorly developed and in some regions completely absent

The studies thus far reviewed appear to answer the questions when and how the ductus closes, but they have raised quite another How is the closure timed so that the ductus arteriosus narrows and remains so only after the ventilation of the lungs is established and yet may be free to relax again at times during the critical stages of the transition from placenta to lung? There appear to be at least three mechanisms by which the contraction of the musculature of the ductus might be timed and wrought to coincide with the establishment of the ventilation of the lungs, *i e*, nervous action, by blood born stimuli, and finally mechanical forces The first possibility—that of nervous action—was presented and supported with the observations of Barcroft *et al* (16) They found that stimulation of the peripheral end of the left vagus in guinea-pig fetuses near term produces a blanching of the exposed ductus and a slowing of the heart Stimulation of the right produced no visible effect on the ductus, though it did slow the heart Upon these observations the suggestion was advanced that the effect of vagal stimulation is a direct one upon the ductus muscle

That there are nerve endings in the ductus of the guinea-pig fetus has been established by Kennedy and Clark (34) but they are not numerous nor are they obviously motor in character Boyd (22) has recently studied intensively the nerve supply to the ductus in a series of fetal forms, including the rabbit, pig, mouse, cat, dog, rat and human Boyd finds that there are nerve fibers in the ductus musculature, which he suggests may be motor in character, but he makes no statement as to their origin I have examined serial sections of the ducti of a large number of sheep embryos and fetuses ranging between 26 days and full term The material had been impregnated by Ranson's pyridine silver method Known nerve fibers and their endings were satisfactorily impregnated Sensory endings of the "depressor" type were found in the adventitia near the aortic end but no fibers were found penetrating to the muscle layer or terminating in motor type endings These studies offer little support for the view that the vagal fibers end on, or directly excite, the ductus musculature

The question Can the ductus be functionally closed by direct nervous action,

remains unanswered, but the more important question Is direct nervous action essential to bring about the closure of the ductus, appears to draw a negative answer from the studies of Kennedy and Clark (35) on the guinea pig These investigators have apparently severed every known and suspected nerve path between the central nervous system and the ductus Constriction of the ductus, despite these operations, followed inflation of the lungs with oxygen through a tracheal cannula As a result of their studies on the denervated ductus, Kennedy and Clark (35) explored the possibilities that its musculature was stimulated by blood-borne substances In view of their observation that inflation of the lungs with air or oxygen did result in narrowing of the ductus whereas inflation with nitrogen did not, oxygen appeared as a substance, blood borne, that might be effective To test this possibility bubbles of pure oxygen were introduced into the umbilical vein, a procedure which was promptly followed by the closure of the ductus in four fetuses Kennedy and Clark (35) suggest the possibility that increased oxygenation of the blood may be the effective stimulus, the oxygen presumably acting directly on the muscle in the ductus There are, however, certain aspects of these experiments that leave open the possibility that the oxygen was acting not on the ductus but elsewhere, perhaps on the brain

Oxygen bubbles introduced into the umbilical vein would be taken up by the blood stream, a part of which, if conditions were normal, would reach the ductus only after being diluted by a greater quantity of blood from the superior cava, or the other part only after being forced through the upper circulation having first reached the heart through the foramen ovale Then, too, there is the objection that the ductus appears to close normally at a time when the oxygen content of the blood is below what it was *in utero* with the placental circulation intact For example A lamb after birth by caesarian section may require some hours to get its blood 90 per cent saturated with oxygen (17) yet *in utero*, before the caesarian section was undertaken, its blood may have been saturated to that extent (17) *In utero* the ductus is open, and it certainly is closed in lambs before the oxygen content of their blood returns to the intra uterine level Nevertheless, the inherent plausibility of the suggestion of Kennedy and Clark and their evidence supporting it are such that it cannot be set aside by these objections Their suggestion was put to a further test in the following manner Arrangements were made to allow warm, whipped sheep's blood saturated with oxygen, to flow into the jugular vein of a full term lamb The lamb was not removed from the uterus, the neck and vein were exposed through a narrow slit in its wall The placental circulation remained intact After 100 cc of blood had entered the jugular, the most direct route to the ductus, 10 per cent formalin was injected via the same cannula and the fetus killed Six experiments were carried out In no one of the six fetuses was the ductus found constricted or even perceptibly narrowed as judged at post mortem (Barron, unpublished) Unless the ductus closed and reopened again when the formalin attacked it, these experiments suggest that an increase in the oxygen content of the blood perfusing the ductus is not sufficient in itself to cause the contraction of the muscle in its walls

The possibility that substances other than oxygen may stimulate the ductus musculature does not appear to have been extensively investigated Kennedy and Clark (35) observed that 1 cc of 1/10,000 adrenalin injected directly into the fetal heart was followed by the closure of the ductus 3 minutes and 1 second after injection started

There remains to be considered the view that the ductus closes mechanically There appear to have been some in the past who have regarded the closure of the ductus as due to pressure upon it, or to stretching or other mechanical forces as a result of inflation of the lung and competition for space in the thorax Such factors may have a rôle but they can no longer be regarded as essential in view of the demonstration of Barcroft *et al* (16) and Kennedy and Clark (35) that the ductus closes when the chest is open

But there is yet another mechanical possibility, one outlined by Zeigenspeck (54), *i e*, that the musculature of the ductus like that of the umbilical vessels is in a tonic state and that the lumen is kept open by the pressure of the blood in it, that pressure offsets the tendency of the ductus to contract Any fall in pressure in the ductus according to this view would remove the balance of forces and permit the ductus to narrow This theory does not appear to have been put to experimental test but there is some evidence to strengthen its probability Hamilton *et al* (29) have clearly shown that the systolic pressure in the right ventricle does fall as breathing begins in the rabbit and this fall would appear to be a necessary consequence of breathing in any other fetal type A fall in the right systolic pressure would reduce the pressure in the ductus Flow from the aorta, the region of higher pressure after breathing is begun, is prevented in the rabbit and dog by valve-like folds at the aortic end of the ductus A permanent reduction in pressure is thus established in the ductus which may permit the musculature to contract Such a mechanism appears to underly the closure of the umbilical veins Its presence elsewhere would not be surprising

There is, however, no valve in many forms, for example, the guinea pig (35), sheep (Barron, unpublished) and the human (29) at the aortic end of the ductus which would prevent blood passing from the aorta into the ductus and so maintaining the pressure in the lesser circulation or even raising it, but it has not been established that the pressure does not fall, normally, sufficiently to permit the ductus to narrow Certainly this possibility is an attractive one for it would offer a simple explanation for the close correlation between the caliber of the ductus and the circulation time through the lung, and would permit an understanding of the failure of the ductus to close in cases of aortic stenosis Against it there is the observation of Clark and Kennedy (35) that inflation of the lungs with nitrogen is not followed by closure of the ductus in the guinea pig, though when the lungs are inflated with oxygen it does so The possible effects of nitrogen on the heart and on the systolic pressure in the right ventricle are unknown

In summary, there is at present no satisfactory explanation of the mechanism initiating the closure of the ductus One may perhaps be permitted to surmise that the explanation, when it is discovered, will be based on simple hemodynamic considerations

*The functional closure of the foramen ovale* There is general agreement that the functional closure of the foramen ovale is accomplished by the apposition of its valve, the septum primum to the septum secundum or interatrial septum (see fig 2) These two structures would appear to be separated when the pressure on the right side of the septum primum exceeds that on its left and approximated when the pressures are equal or reversed No studies of these pressures prior to the onset of respiration or the impairment of the placental circulation appear to have been made, but so long as a part of the return from the post-caval channel enters the left atrium the pressure in that vessel would appear to be equal to or to exceed the filling pressure via the lung capillaries A fall, therefore, of the pressure in the post-caval channel or a rise in the filling pressure could serve to close the foramen ovale functionally

A fall in the pressure in the post-caval channel might be brought about by increased resistance in the capillary bed of the placenta or by constriction of the ductus venosus Both changes occur at birth The expansion of the chest and the entrance of air into the lungs may be expected to increase the filling pressure of the left atrium for they reduce the pressure against which the right heart works—from atmospheric to something between atmospheric and intrathoracic—and the lateral pressure on the lung capillaries by replacing solid lung tissue with alveolar air

Just which one of these changes in pressure or combination of them brings about the initial closure of the foramen ovale has not been determined At a normal birth the placental circulation is impaired and the fall of the pressure in the posterior cava must precede the ventilation of the lungs But at delivery by caesarian section the circumstances may be reversed In the one lamb, delivered and studied radiographically by Barclay *et al* (7), the foramen ovale was closed within four minutes after breathing began, while the ductus arteriosus was still patent and before the umbilical cord was tied In this case a rise in pressure on the left side of the septum primum was probably responsible for the closure This observation makes it clear that the functional closure of the foramen occurs suddenly and not slowly over a long period of time as some investigators have supposed There is indirect evidence to support this conclusion Barcroft, Kramer and Millikan (18) observed that the blood in the carotid of a lamb delivered by caesarian section can become completely saturated with oxygen five minutes later if the lamb be given pure oxygen to breathe, a circumstance which precludes any appreciable degree of contamination of the blood in the left atrium

*The closure of the ductus venosus* With the ductus arteriosus and the foramen ovale closed the substitution of the lung for the placenta as a source of oxygen is completed There remains but the closure of the ductus venosus, the substitution of the fetal gut for the placenta as a source of nutrition, before the transition from the fetal to the adult circulatory pattern is completed

The closure of the ductus venosus does not appear to have attracted the interest that has centered about its counterpart, the ductus arteriosus Yet its persistence as a patent channel would result in the adult in a condition similar to that attained by Eck's fistula Anatomical studies (48) (Barron, unpublished)

indicate it is closed by the day following birth The only records of its functional closure are those mentioned above, the radiographic records of Barclay *et al* (8) In the case they describe in greatest detail, the ductus was opening and closing ten minutes after delivery by caesarian section, the umbilical cord was undivided and ventilation of the lungs was prevented by a nose bag put on *in utero* These are not conditions which would appear to permit the closure of either the ductus arteriosus or the foramen ovale, in short they appear to resemble conditions *in utero* more than those of free birth There may be room for a reasonable doubt that these constrictions in the ductus venosus were a part of the activity associated with the delivery of the fetus from the uterus Nevertheless there is good reason to suppose that the final closure at birth is wrought by the same mechanism

In summary, the available evidence indicates that the ductus arteriosus and the ductus venosus are closed functionally within a few minutes after birth by the action of sphincter muscles appropriately placed It does not permit any conclusion as to how the contraction of these two sphincters is evoked and timed with other aspects of birth Similarly the evidence indicates that the closure of the foramen ovale may take place abruptly and immediately after birth but the forces responsible for its closure have not been described

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# PHYSIOLOGICAL REVIEWS

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## THE ANTICOAGULANTS EFFECTIVE IN VIVO WITH SPECIAL REFERENCE TO HEPARIN AND DICUMAROL[1]


ARMAND J. QUICK

*Department of Pharmacology, Marquette University School of Medicine, Milwaukee*


The successful employment of heparin and dicumarol in the treatment and prevention of thrombosis is largely responsible for the present interest in the anticoagulants. Aside from their utilitarian potentialities, these agents are of special significance to the student of physiology since they furnish important clues concerning the mechanism of the coagulation of the blood. It will be the purpose of this review to present the chemical and physiological action of the principal substances that inhibit the coagulation of blood with only incidental reference to their clinical application. No extensive historical discussion is deemed necessary since the writer summarized much of this material in his recent monograph (77), and Wöhlisch (114), Mason (65), Jorpes (47), Best (6), Prandoni and Wright (73) have reviewed both the older and more recent literature.

THE NORMAL ANTITHROMBIN OF THE BLOOD. It has long been known that plasma or serum will inactivate large amounts of thrombin. There is no evidence that this antithrombic substance in blood is an anticoagulant, since Volkert (107, 108), as well as earlier investigators, have found that changes in its concentration have no demonstrable effect on the coagulation time. The writer (77) has postulated that the affinity of thrombin for fibrinogen is greater than for the plasma antithrombin and as a consequence little thrombin is combined and inactivated by the antithrombin before all the fibrinogen is converted to fibrin.

The writer's observation that the antithrombin resides almost entirely in the albumin fraction has been verified in various laboratories (2, 3, 33, 101, 116), but all attempts to isolate the active constituent have failed. Wöhlisch and Köhler (117) report that the antithrombic potency of serum albumin can be eliminated by extraction with ether or chloroform, and Grüning (39) recently stated that a substance possessing full antithrombic activity can be recovered in the extract. He concluded that the antithrombin is not a protein but a fat or lipoid closely associated with the protein. The fact that the potency of the extract is destroyed at 50°C. makes one hesitate to accept that it is a simple lipoid. Until more concrete information is available, it may be well to continue calling it Albumin X.

Attempts to determine variations in the antithrombin concentration of blood are not new as Astrup and Darling (3) point out, but due to unsatisfactory methods, most of the earlier findings must be regarded as qualitative. Astrup and Darling (3) have developed a quantitative method which consists in incubating

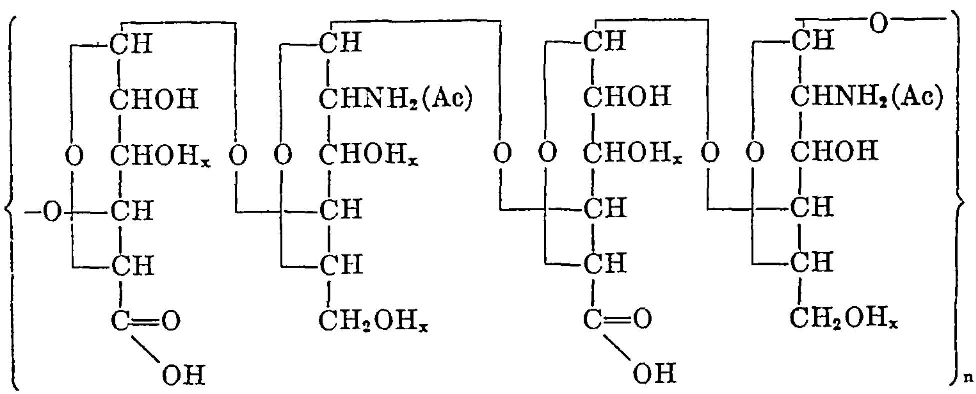
[1] Dicumarol is the name adopted by Link and his associates and is used throughout this review.

1 cc of thrombin of standard strength with varying amount of serum (0 to 0 2 cc ) at 37°C for 15 minutes At the end of this time, the clotting power of the mixture is determined by allowing 0 1 cc of the mixture to react with 1 cc of fibrinogen solution From these data the amount of thrombin consumed can be calculated

With this method Volkert (107, 108) has found that the normal antithrombin gradually rose to a maximum in about 14 days after injecting soluble proteins such as egg albumin and horse serum into rabbits and dogs It appears unlikely that this is an immunological response since a similar increase was observed when gelatin, starch and other substances without antigenic properties were injected, whereas no response occurred when corpuscular antigens, such as human erythrocytes, were given intravenously Curiously, India ink caused a transient drop as did liver injury from chloroform and carbon tetrachloride, while ligation of the common duct increased the antithrombin Dyckerhoff and Marx (32) also have found an increase of antithrombin in jaundice Wilson (112), employing a method essentially the same in principle as that of Astrup and Darling but differing in the details of the procedure, has found that the antithrombin content of plasmas of man and the common laboratory animals show no striking differences

The significance of antithrombin in blood is not known Wöhlisch (115) voices the same opinion expressed by the writer that it probably serves as a protection against small amounts of thrombin formed intravascularly One of the perplexing problems is the relation of this normal antithrombin to heparin, which will be presently discussed

HEPARIN *Chemistry* It is not known whether heparin is a single substance or a group of closely related compounds Jorpes and Bergström (50) and Charles and Todd (22) agree that heparin contains glucosamine, a uronic acid, which has not been satisfactorily identified, and several sulfuric acid groups in ester linkage The latter investigators propose the following formula for beef heparin



The *x* indicates the probable position of the sulfate groups It is not known how completely the amino groups of the glucosamines are acetylated In the formation of salts, such as that of barium, the hydrogens of the sulfate groups rather than of the carboxyl groups are believed to be replaced

It can be seen that the formula closely resembles mucoitin sulfuric acid Apparently minor changes in the molecule which may not be detected chemically can markedly change the biological activity Jaques, Waters and Charles (46) have found that dog heparin has an activity of 240 units per mgm , beef of 100, pork 44, and sheep 23 No chemical differences could be detected All contained the same percentage of sulfur and nitrogen, and had the same optical rotation Jorpes (48, 49) also noted the high activity of dog heparin, but he observed variations in the sulfur content Kuizenga and Spaulding (54) recently recorded that the barium salt of beef lung heparin containing 100 units per mgm can be separated into two fractions, one of which has a potency of 125 units per mgm

These results suggest that heparin may not be a single substance with a fixed activity but a mixture of compounds having a mucoitin polysulfuric acid structure, but differing perhaps in the number of sulfuric acid radicals and in other chemical groups

*Action* The behavior of heparin in the blood differs from that observed in a test tube when allowed to react with purified reagents Heparin by itself is not an anticoagulant In a mixture of purified fibrinogen and thrombin it has almost no inhibitory action and likewise it shows no retarding or blocking effect on the conversion of prothrombin (precipitated from plasma) to thrombin (74) It should also be emphasized that heparin is not neutralized by thromboplastin, a finding that is repeatedly disregarded even in the current literature

The key to the physiological action of heparin appears to be its strongly acidic property by virtue of which it forms stable salts with many proteins (37) In this reaction the physical and chemical properties of the proteins such as isoelectric points, solubility and electrophoretic patterns are altered If the protein has enzymatic or other biological activity, one can postulate that heparin may influence, i e , intensify or inhibit these properties

Jaques (44) has studied the reaction of heparin on a number of proteins including casein, gelatin, protamine and the dye, toluidine blue He found in agreement with Fischer's earlier postulate that a stoichiometric relationship exists between heparin and the protein

$$\text{Protein} + \text{heparin} \rightleftarrows \text{protein heparin}$$

or

$$\frac{C_{\text{protein}} \times C_{\text{heparin}}}{C_{\text{protein heparin}}} = K$$

A strongly basic protein such as the protamine, salmine, will form a heparin salt which is little dissociated, while a compound like gelatin when combined with heparin readily undergoes dissociation Certain factors such as the pH, variations in the anion and cation concentration, and probably the presence of certain lipoids may influence the dissociation of the various protein heparin complexes

*The anti-thrombic action of heparin* The coagulation of fibrinogen by thrombin is prevented by heparin provided a co-factor is present This latter agent is found in the plasma, specifically in the albumin fraction The fact that this co-

factor loses its antithrombic activity in conjunction with heparin when heated to 67°C , suggests that it is a protein similar or identical with a fraction of serum albumin (76) Ziff and Chargaff (119) found that all albumin fractions displayed some activity except the crystalline product Later these authors (21) observed that serum albumin separates into three fractions electrophoretically and that the fast and middle fractions are active as heparin co-factors, while the slow fraction is not

One can postulate that heparin unites with a fraction of serum albumin, which for convenience can be designated Albumin $X_1$ and the resulting complex, Albumin $X_1$-heparin has the ability to bind thrombin and therefore is a true and powerful antithrombin Heparin also combines with other proteins, for Chargaff and his associates (21) have obtained evidence that it unites even with plasma globulins When heparin is added to blood, it will be distributed between the various plasma proteins Theoretically, qualitative and quantitative variations in the proteins may influence the anticoagulative effectiveness of heparin, but this has received no extensive study either experimentally or clinically The findings of de Takats (102) that the coagulation time response to injected heparin shows marked variations, may perhaps be a manifestation of plasma protein alterations

When a strongly basic protein like protamine is added to blood made incoagulable with heparin, the antithrombic activity is immediately lifted (20) Presumably the heparin is removed from Albumin $X_1$ and combines with protamine, for the dissociation constant of protamine-heparin is smaller than that of the Albumin $X_1$-heparin complex

Both the normal antithrombin and the co-factor of heparin are constituents of serum albumin, both are inactivated at 67°C and both are absent in crystalline albumin Quick (76) postulated that the two are identical and that when heparin is united with the normal antithrombin its action is so intensified that it becomes a powerful anticoagulant Seegers and his associates (90, 92) reached a similar conclusion, namely, that thrombin is destroyed by the antithrombin of the plasma and that heparin merely accelerates the speed of the reaction but does not increase the capacity of plasma to inactivate thrombin They consider heparin to be essentially a catalyst On the basis of this conclusion Seegers and Smith (91) propose a quantitative method for the assay of antithrombin (i e , of the co-factor)

Astrup and Darling (2) reject the concept that normal antithrombin and the heparin co-factor are identical They state that in purifying serum albumin, they obtained fractions that possessed no antithrombic properties but were activated by heparin, and vice versa, fractions that were antithrombic were not further activated by heparin The actual experimental procedures are not recorded They mention that the co-factor in ox blood is inactivated by heating at 56°C for 5 minutes while the antithrombin concentration is not reduced Further investigation is needed to solve this important problem concerning the relationship of antithrombin to the co-factor of heparin

*The antiprothrombic action of heparin* Howell and Holt (41) in describing

heparin and its action reported experiments which indicated that this agent inhibited the conversion of prothrombin to thrombin This view was generally accepted until it was shown that prothrombin isolated from plasma is readily converted to thrombin in the presence of heparin (66, 74) Brinkhous and his associates (12) confirmed the observation that heparin alone is not an anti prothrombin, but found that in the presence of serum it completely inhibits the conversion of prothrombin to thrombin They conclude that heparin acts only in combination with a plasma factor but from their data, they were unable to say whether the action is antiprothrombic or antithromboplastic (Since excess thromboplastin does not clot plasma made incoagulable with a minimal amount of heparin, it seems unlikely that heparin plus co-factor constitutes an antithromboplastin ) They offer no information concerning the nature of the co-factor except that it is not dialyzable Ferguson and Glazko (35) by means of a rather involved type of reasoning conclude that heparin has a direct action and that the first effect is antithromboplastic i e , an inhibitory action on the tryptase-like thromboplastic enzyme, while the second effect is on the prothrombin molecule In addition to this intrinsic action of heparin, a co-factor found in crude plasma albumin markedly potentiates the anti prothrombic action

Progress in unravelling this intricate problem is contingent on learning more about the nature of prothrombin and the manner in which it occurs in unaltered blood and in decalcified plasma Since evidence has been obtained that prothrombin consists of two components combined with calcium and that removal of the latter ion causes a disruption of the prothrombin complex (78), one can justifiably question whether the inhibitory action of heparin in blood is necessarily the same as in decalcified plasma

*Heparin and platelets* It has been recognized since the time of Bürker (1904) that all agents that inhibit coagulation also prevent the agglutination of platelets The action of heparin in stopping the clumping of platelets is presumably not directly on these cells but is the result of its anticoagulant property Baronofsky and Quick (5) found that human blood to which 0 25 mgm of heparin per cc was added showed no clumping of platelets or any diminution in their number The finding of Copley and Robb (25) that heparin causes a decrease of the platelet count both in vitro and in vivo could not be verified

The starting point of a thrombus is a small mass of agglutinated platelets, and the prevention of this clumping is probably the primary action of heparin in its prophylaxis against thrombosis The classical work of Best and his co-workers (7) who studied platelet agglutination in the living animal by means of a glass cell (with a transverse scratch on its inner surface) which is connected between the carotid artery and the jugular vein, has done much to clarify the concept of thrombosis and to place therapy on a scientific basis Interestingly it was found that a dose of heparin which immediately raised the clotting time of a dog to 6 hours, did not prevent the agglutination of platelets (97) To accomplish the latter, large doses (over 300 mgm /kgm of body weight) were necessary and the effect required 15 to 50 minutes to become manifest It might be stated that *in vitro* the action of heparin is immediate

*Heparin in peptone and anaphylactic shock.* In both types of shock three things occur: a marked diminution of platelets, a liberation of histamine and an outpouring of heparin into the blood stream, which may be sufficiently massive to bring about complete incoagulability. The author in his monograph attempted to correlate these three effects and postulated that heparin served as a defense agent. Unfortunately this speculation was at best premature and has not been borne out by facts. At present there is little evidence to support any relationship between platelets, histamine and heparin. Histamine when injected does not cause any significant decrease in platelets. This observation was reported by Kinselle et al. (52) and confirmed in the writer's laboratory, thus invalidating earlier reports to the contrary. Heparin does not protect rabbits or guinea pigs against anaphylactic shock induced by horse serum. In rabbits the only manifestation following a shocking dose may be a precipitous fall in platelets, without any other gross concomitant signs, and this decrease in platelets is not prevented by excessively large doses of heparin. The depletion of platelets is caused by a marked agglutination immediately after the shocking dose followed by a rapid removal of the platelet clumps. The effect is on the circulating platelets, and is temporary, since full restoration in number occurs in 30 minutes. (Unpublished results.) Since heparin does not appear to inhibit the agglutination of platelets in shock but does prevent platelet clumping due to contact with a foreign or rough surface, one must question whether the two types of platelet aggregation are identical.

Dragstedt and his group (31) have reported that heparin inhibits in vitro the release of histamine from cells to the plasma brought about by trypsin, proteose or a specific antigen. The amount of heparin needed is higher than that required to prevent coagulation. Whether this observation is related to the finding of Landis et al. (56) that the vasoconstricting effect of defibrinated blood can be prevented by heparinizing blood or plasma is not known.

With the actual isolation of heparin from the blood of dogs in anaphylactic shock by Jaques and Water (45) it is now certain that this substance is responsible for the incoagulability of the blood in peptone and anaphylactic shock. As further evidence they demonstrated that the liver after shock contained less heparin than the normal liver, and by histological studies following Wilander's toluidine-blue staining reaction they showed damage to the mast cells, which contain heparin in the form of metachromatic staining granules. The article of Best (6) should be consulted for the work on the mast cells and their relation to heparin. The mechanism and the stimulus responsible for the discharge of heparin from the mast cells into the circulation is still an unsolved problem.

The question whether heparin protects against anaphylactic shock remains unanswered. Recently Macht (64) again reported success in preventing anaphylactic shock with heparin while others including the writer (unpublished results) and Jaques (44) failed to demonstrate such protection. It is possible as Jaques states that it may be difficult to reproduce the necessary condition to demonstrate this inhibitory action of heparin, and he suggests that the action may be explained by assuming that heparin uniting with the sensitizing protein

so changes the antigenic property that it can no longer elicit an anaphylactic shock

*The standardization of heparin* Although no unit of heparin potency has been officially accepted, the crystalline barium salt of the Connaught Laboratories of the University of Toronto can well serve as a standard and the proposal that the unit be defined as 0 01 mgm of this salt seems desirable Such a unit is about five times larger than the original Howell unit

Various methods for assaying heparin have been proposed Howell's method consists in determining the amount required to keep 1 cc of cat blood liquid for 24 hours under standardized conditions This type of procedure has been very useful, but is time-consuming, cumbersome, and subject to various errors

A second method is based on finding the minimum quantity of material necessary to prevent the coagulation of recalcified oxalated plasma Foster (38) and Kuizenga and his co-workers (53) have recently described such methods MacIntosh's (61) method is similar except that he adds an excess of thromboplastin, thus eliminating variability of this factor

A satisfactory method (77) consists in adding the heparin to oxalated plasma (preferably human) and then titrating with increasing amounts of a standardized thrombin solution, using the clot as the end point The unit of heparin is expressed in terms of its capacity to neutralize a fixed amount of thrombin This method has the advantages of simplicity and of yielding reproducible results

Jaques and Waters (45) have observed that 1 9 mgm of protamine (salamine) neutralizes 1 mgm of heparin in dog blood, and from this finding have developed a quantitative procedure for titrating the quantity of heparin in the blood during anaphylactic shock

MacIntosh (61) has developed a colorimetric method based on the observation that heparin reacts with toluidine blue forming a complex which can be removed with petroleum ether while the unchanged dye remains in solution The heparin can be calculated from the loss of dye Copley and Whitney (27) have likewise employed toluidine blue as well as Azure A for determining heparin colorimetrically They report that 1 unit of heparin (Connaught Laboratory) decolorizes 15 gamma of 100 per cent toluidine blue dye content This unit equals 25 4 gamma of toluidine blue Nu-3 or 17.2 gamma of Azure A Naz-7 A comparison of the anticoagulant and colorimetric methods shows discrepancies which may be attributed to inherent errors in both types of assay

The urgent need now is not so much a better method for assaying heparin, but procedures to determine the co-factors of heparin Since plasmas of various species are apt to vary in their content of these factors, it is to be emphasized that this should be taken into consideration in the interpretation of results Even differences in thrombin or thrombin like agents may give rise to misleading results Thus Rigdon and Haynes (83) found that heparin does not inhibit the clotting agent, coagulase, produced by certain strains of staphylococci This does not mean necessarily that heparin is ineffective, it may be due to the lack of a suitable co-factor

*Physiological significance* The very fact that the organism possesses a large

store of heparin seems indisputable evidence that it has an important function, nevertheless, its rôle in the body economy has not been found It is still a moot question whether normal blood contains any free heparin If it does, the amount is too small to be unequivocably demonstrated Certain it is that the fluidity of blood can be as convincingly explained by hypotheses that exclude heparin as by those that center about it The purpose of heparin in anaphylactic shock is, as already stated, quite obscure To the best of the writer's knowledge, the literature contains no record of any instance in which an abnormal amount of heparin has been found in human blood The finding of Macht (64) that heparin lowers the toxicity of ouabain and digitalis suggests that it functions as a protective agent, since these substances are reported to increase the coagulability of the blood

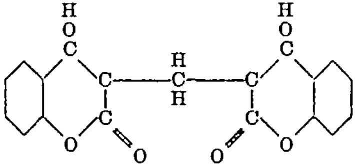
Excess amounts of heparin in the blood are quickly reduced A portion of the compound is excreted in the urine, as high as 10 to 35 per cent when 200 units per kilogram of body weight are injected into a dog (26) A large amount is not accounted for, but the recent work of Jaques (43) indicates that tissues contain an enzyme, heparinase, which inactivates heparin The prompt removal or destruction of heparin has necessitated continuous intravenous administration when used clinically but Loewe et al (60) have found that a prolonged anticoagulant action can be obtained by injecting subcutaneously heparin incorporated in a slowly absorbing medium

ANTITHROMBOPLASTIN Antithromboplastins have been proposed from time to time, but usually received but passing notice since the evidence on which the existence of such inhibitory agents was based was usually not convincing During the past year Tocantins (103, 104) has demonstrated that thromboplastin when incubated with plasma is either destroyed or inactivated In hemophilia this inactivation is marked, and he believes that this accounts for the delayed coagulation in this disease He postulates that the thromboplastin is destroyed as fast as it is liberated from the platelets, therefore not enough can accumulate to initiate coagulation In a later publication Tocantins (105) reports results which indicate that the antithromboplastic activity is directed toward the lipid moiety of thromboplastin, i e, against cephalin Interestingly, Russell viper venom is not inactivated by the plasma antithromboplastin In some respects, protamine when added to plasma acts as the naturally occurring inhibiting agent

DICUMAROL (3,3′ METHYLENE-BIS-(4 HYDROXYCOUMARIN) The tempo of modern science is dramatically illustrated by the solution of the hemorrhagic disease caused by spoiled sweet clover hay Two decades after the disease was discovered and described by Schofield (88, 89), Link and his associates (17, 18, 19) not only successfully isolated and identified the compound responsible for the hemorrhagic diathesis, but also described a practical method of synthesis (98) so that the drug immediately became available for both experimental and clinical purposes The achievement of Link was in no small measure expedited first by the pioneer studies of Roderick (84, 85, 86) who demonstrated that a marked drop of prothrombin occurs, and secondly by the investigations of the writer (75) who reported the first quantitative studies on the effect of toxic

sweet clover hay on the prothrombin level and developed a simple and reliable procedure for determining prothrombin which was adaptable for assaying the hemorrhagic factor in sweet clover hay

*Chemistry* The structure of the toxic principle in spoiled sweet clover is



3,3′ methylene-bis-(4-hydroxycoumarin)

The compound is a colorless crystalline solid, relatively insoluble in water, acids and in the common immiscible solvents With strong alkalies it forms soluble salts The sodium salt solution gels on standing and slowly turns brown probably due to oxidation A high alkalinity is required (<pH 8) to keep dicumarol in solution, which is somewhat of a disadvantage for intravenous administration Although the compound is insoluble it is readily absorbed from the gastrointestinal tract and even to some extent when administered rectally (69) The compound is optically inactive When fused with potassium hydroxide, each molecule yields two molecules of salicyclic acid (98)

*The action of dicumarol* The work of Roderick in 1931 (85) and of the writer in 1937 (75) established the fact that the principle of toxic sweet clover hay reduces the prothrombin of the blood and this has been repeatedly verified since dicumarol became available in 1941 Heretofore prothrombin has been considered as a single substance, but recently the writer (78) has presented evidence showing that this agent is composed of two components (A and B) which are linked through calcium Component A gradually disappears from stored plasma whereas component B is stable It is component B which decreases in dicumarol poisoning and it is this factor which also appears to be diminished in vitamin K deficiency This suggests a relationship between dicumarol poisoning and avitaminosis K

The mechanism whereby dicumarol reduces the prothrombin (or more correctly component B) of the blood is not fully understood, but all evidence points to a depression of the synthesis of prothrombin i e causing an inability to utilize vitamin K rather than to a direct action on prothrombin This view is expressed by Bingham, Meyer and Pohle (8) and others (1, 42, 72) Witts (113) points out that there is a basic similarity between the structure of 4-methyl 1,4 naphthoquinone and 4-hydroxy coumarin and suggests that dicumarol might act by interfering with the utilization of vitamin K by the liver

It is not unlikely that dicumarol poisoning is basically similar to carbon monoxide intoxication Neither agent causes any demonstrable morphological changes Both impair one vitally important function without disturbing the other activities of their respective organs Carbon monoxide blocks the oxy-

gen carrying power of hemoglobin, but the other activities of the blood are not directly affected, dicumarol interferes with the synthesis of prothrombin, but does not disturb the other functions of the liver In both intoxications function is restored when the noxious substance is removed, and removal is hastened by the agent which the toxin excludes from the cell oxygen in carbon monoxide poisoning and vitamin K in dicumarol intoxication The most serious sequellae in both types of poisoning come from tissue anoxia, in the one, the anoxia is direct, in the other it is secondary to extensive hemorrhages and subsequent anemia

*Quantitative action of dicumarol on the prothrombin level* Few satisfactory quantitative studies of the action of dicumarol on the prothrombin level of the blood have been reported In most studies only the prothrombin time is given, but due to the variability of the procedures for determining prothrombin and the differences in activity of the thromboplastin employed, it is not possible to calculate accurately from these data the changes in prothrombin It is found by nearly all workers that after a single dose, the prothrombin slowly decreases, reaches its lowest point usually in 48 to 96 hours and again becomes normal in about a week Bollman and Preston (11) state that 10 mgm of dicumarol per kgm of body weight given intravenously to dogs caused the prothrombin to fall to about 15 per cent in three days Overman et al (70) recorded that a single dose of 2 5 mgm lowered the prothrombin of a 250 gram rat to 22 per cent of normal in 24 hours In another paper Overman and his co-workers (72) state that a prothrombin time of 10 minutes has been induced in dogs by a single large dose of dicumarol Such a result must certainly be the exception rather than the rule

The writer (78 and unpublished results) has found that the prothrombin decrease follows a fairly uniform pattern in both dogs and rabbits The prothrombin level falls to approximately

20 per cent of normal in 24 hours
5 per cent of normal in 48 hours
$1\frac{3}{4}$ per cent of normal in 72 hours
1 per cent of normal in 96 hours

A critical minimum dose is necessary to effect these results, but even greatly increasing the dose above this will not hasten the drop in prothrombin The pattern remains the same whether the drug is given orally or intravenously Certain animals are more resistant to dicumarol, a fact which Link (17) found early in his work The writer has made the interesting observation that chickens are particularly resistant to dicumarol and recover very promptly from its effect

The findings are best explained by the hypothesis that dicumarol inhibits the enzymatic mechanism which produces prothrombin The decrease observed actually represents the body's consumption or destruction of prothrombin As the enzymatic system is freed of the toxin, it gradually regains its capacity to synthesize prothrombin The beneficial action of vitamin K, which has been reported, can be explained by assuming either that it can replace the toxin,

or that the synthesizing cells remaining unaffected can manufacture more prothrombin when an excess supply of vitamin K is available

*Quantitative determination of prothrombin* Since much of the work on dicumarol depends on the determination of prothrombin, a critical discussion of the one-stage method is indicated Unfortunately the two-stage method of Smith has not been used extensively so that comparisons of results obtained by the two methods cannot be made McGinty and his associates (62) report that after giving a dog two doses of dicumarol (14 mgm. per kgm of body weight) the prothrombin determined by the two-stage method dropped to below 20 per cent of normal in 48 hours This and their other results agree in general with the writer's data obtained with the one-stage method

The British investigators have been particularly critical of the one-stage method and have condemned it in no uncertain terms Macfarlane (63) writes "It is clear that Quick's test has broken down completely where the coumarin compound is concerned" Witts (113) is equally outspoken and he proposes changing the name, "prothrombin time" to "accelerated clotting time" Most of the criticism centers about the occasional finding that the coagulation time was shorter than the prothrombin time Such anomalous results have been reported by Bingham et al (8) and by Davidson and MacDonald (29) The latter recorded that on one occasion, the prothrombin time was over 1 hour while the coagulation time was 17½ minutes and the clotting time of recalcified plasma 9 minutes In contrast to such results Townsend and Mills (106) record a case with a prothrombin time of 420 seconds and a corresponding coagulation time of 39 minutes, and later of 566 seconds and 60 minutes respectively The writer has in his unpublished files many similar results, and has never observed a coagulation time which was shorter than the prothrombin time Butsch and Stewart (13) have reported a series of cases with both prothrombin and coagulation times and in every instance the latter was many times more prolonged than the former

It should be obvious that discordant results can come from faulty technique equally as readily as from basic defects of the procedure Accurate timing of clot formation becomes difficult when the prothrombin falls below 1 per cent of normal Poor thromboplastin is undoubtedly the cause of much inaccuracy The writer has explicitly described the method for preparing a highly active thromboplastin with a remarkable constancy of potency There is no longer any valid excuse for reporting prothrombin data based on thromboplastin which does not yield a prothrombin time of 12½ seconds for human plasma and 6 seconds for dog or rabbit plasma Russell viper venom which is recommended by Hobson and Witts (40) has not yet been satisfactorily standardized for the quantitative estimation of prothrombin Particularly confusing is the fact that the activity of the venom is markedly potentiated by lecithin It is difficult to determine from the literature whether the venom should be used with or without lecithin in the test and how the results should be interpreted

There is no evidence that Link's modification (17) of the original one-stage method, which is essentially running the prothrombin time on plasma diluted

with 7 volumes of saline solution is more accurate The claim is made that it is more sensitive to small changes of prothrombin, but since the range in which the greatest accuracy is usually desired especially for clinical purposes is below 20 per cent of normal, increasing the prothrombin time by dilution offers no advantage, and is actually undesirable for low prothrombin values

The best evidence for the reliability of the one-stage method is the finding that when a dicumarinized animal is given a transfusion of normal blood, the calculated expected rise of prothrombin and the actual increase found agree remarkably well This experiment is routinely demonstrated to our students

*Vitamin K and dicumarol* The studies of the action of vitamin K on the hypoprothrombinemia induced by dicumarol are difficult to evaluate The writer in 1937 (75) believed he had evidence that alfalfa in the diet of rabbits counteracted the action of toxic sweet clover, but Smith (96) failed to find that this material had any protective action Campbell et al (17) likewise found that 2-methyl 1,4-naphthoquinone or natural vitamin K did not prevent the reduction of prothrombin by toxic sweet clover Bingham and Meyer with Pohle (8) and later with Axelrod (67) found that the hypoprothrombinemia was not influenced by synthetic vitamin K Allen, Barker and Waugh (1) as well as Wright and Prandoni (118) and Lehmann (57) reported similar observations Bollman and Preston (11) noted that dogs when fasted were more susceptible to dicumarol, but this disappeared when vitamin K was administered Both Lehmann (58) and Townsend and Mills (106) have reported satisfactory clinical responses from transfusions plus vitamin K in severe hemorrhagic conditions produced by dicumarol, but the prothrombin level was not followed accurately enough to allow interpretation of these results

King and his associates (51) presented findings which suggested some antagonism of alfalfa to dicumarol Overman and his co-workers (70) reported that rats on a diet free of vitamin K showed the maximum susceptibility to dicumarol All forms of vitamin K, natural as well as synthetic, counteracted the hypoprothrombinemia, and the authors express the opinion that the main effect is a stimulation of the recovery process A few months later these workers (71) published further data substantiating the antagonistic action of alfalfa on the effect of single small doses of dicumarol They made the interesting observation that menadione and *l* ascorbic acids when fed at high levels successfully counteracted small single doses (2 5 to 5 mgm ) of dicumarol in rabbits Menadione by itself was effective, and in some rabbits even *l* ascorbic acid alone was antagonistic to the toxic sweet clover agent In all these experiments a single small dose of dicumarol was administered Shapiro and his associates (93) using the same technique have verified the above finding Davidson and MacDonald (29) found no antidotal action of synthetic vitamin K to dicumarol, but later (30) reported that vitamin $K_1$ oxide in large doses (as high as 250 mgm ) prevented or reversed the hypoprothrombinemia produced by dicumarol in humans, but also in these experiments only a single dose of dicumarol was given It seems fairly well established that by rather drastic methods, some antagonism of vitamin K to the action of dicumarol on prothrombin can be demonstrated,

but other factors probably play a part More information and study are needed before definite conclusions can be drawn

*Transfusion and prothrombin response* The writer (75) in his early studies recorded a sharp but transient rise of prothrombin in dicumarinized rabbits after a transfusion of fresh blood This observation has been repeatedly confirmed In view of the recent finding that prothrombin is composed of two components and that factor A disappears when plasma is stored while factor B is diminished in dicumarol poisoning, the use of "banked" plasma should be equally as effective as fresh blood Cahan (16) in one case has obtained an effective response from citrated banked blood whereas Wright and Prandoni (118) found it ineffective in one case Obviously more study is required before any conclusions can be drawn especially since it has even been reported that transfusions with fresh blood were ineffective in some patients (111) McGinty and co-workers (62) have obtained promising results with the intravenous injection of purified prothrombin for correcting the depletion of this agent from dicumarol poisoning

*Effect of dicumarol on the liver* Other than depressing the prothrombin, which is synthesized in the liver, dicumarol appears to exert no other deleterious effect on this organ Animals can be repeatedly subjected to courses of dicumarol poisoning without sustaining liver injury as Link and others have repeatedly observed On autopsy such animals are found to have livers which are entirely normal (8, 11, 15) Liver function tests likewise show no impairment of the organ (1, 14, 29, 118) Even after administering dicumarol continuously for 92 days to a patient, no detectable changes in liver function were observed by Bingham and his co-workers (9)

According to Rose Harris and Chen (87) central necrosis of the liver is fairly common in rats after subjecting them to repeated doses of dicumarol Similar lesions were also occasionally observed in mice and rabbits Richards and Cortell (81) also noted fatty infiltration of the liver and scattered areas of necrosis In guinea pigs the livers were not unlike those observed in vitamin C deficiency Since many of these animals had an advanced anemia, it seems rather probable that the liver damage seen might have been due to anoxia rather than to the direct result of the drug It is a characteristic finding clinically that patients rarely show any untoward symptoms except hemorrhage (4) In animals that receive lethal doses (40 mgm or more per kgm of body weight given intravenously) a dark and congested liver was found (109) but such doses are never given clinically

*Factors influencing the action of dicumarol* It has already been stated that certain animals are more resistant to dicumarol than others Whether normal men differ in their susceptibility is not known, since all reported studies have been made on patients Wright and Prandoni (118) found that age and sex had no influence, and that no significant correlation existed between malnutrition and the frequency of toxic reactions Butsch and Stewart (14), however, record that debilitated and cachectic patients had a more prolonged and a greater elevation of the prothrombin time than did the average patient

To what extent impaired liver function influences the toxicity of dicumarol

is not definitely known Shapiro and his associates (94) noted that patients with cirrhosis showed a decrease of prothrombin when given 50 mgm of dicumarol, whereas patients without liver dysfunction showed no effect from such a small dose Likewise rats given a hepatotoxin such as carbon tetrachloride were found by Richards and Steggerda (82) to have an increased response to dicumarol In bilaterally nephrectomized rats, the prothrombin after a single dose of dicumarol continued to drop until death (82) whereas in a normal rat the prothrombin begins to rise after the second day This experiment illustrates the importance of the kidney in eliminating the toxic principle from the body After anesthetic doses of chloroform, pentobarbital and pentothal, the prothrombin response to dicumarol is not materially different from that of unanesthetized rats (94)

Most interesting are the findings of Field et al (36) that lactating rats are unusually resistant to the action of dicumarol and that this protective period lasts throughout lactation even when abnormally prolonged Perhaps the observation that cows fed toxic sweet clover hay may survive parturition, whereas the newborn calf dies of hemorrhage may be accounted for on the basis of Field's findings Fever experimentally produced in rats caused a heightened susceptibility to dicumarol according to Richards (80) The clinical implications of these findings are obvious It would be interesting to see whether a high intake of ascorbic acid can counteract the animal's increased sensitivity to the drug during fever Since the hypoprothrombinemia due to dicumarol is increased in scurvy both in extent and duration (101a) any condition causing an abnormal consumption of vitamin C is apt to exaggerate the effect of dicumarol

*Dicumarol and capillary fragility* Bingham, Meyer and Pohle (8) stressed that dicumarol causes widespread vascular dilatation of the viscera of dogs especially after massive doses Bollman and Preston (11) report similar findings after large doses (100 mgm per kgm of body weight), and Dale and Jaques (28) likewise state that they have confirmed the result of Bingham et al Cahan (16) has described a case of purpura following dicumarol (100 mgm daily for 32 days) A bleeding time of 13 minutes was found, but clot retraction was normal, indicating that the platelets were not involved Usually the bleeding time is not prolonged even when the coagulation time is greatly delayed (1, 29, 55) Wright and Prandoni (118) emphasize that they could detect no increase in capillary fragility even in frank hemorrhagic cases Why there should be such a discrepancy in results is difficult to understand, but curiously even the reports on as simple a procedure as the sedimentation rate are confusing Allen et al (1) state that the sedimentation rate is routinely increased while Wright and Prandoni (118) find no such change

*Dicumarol in the prevention of thrombosis* The primary objective of much of the work, on dicumarol is its utilization for the prevention of thrombosis The writer in his studies of 1936 observed that heart puncture in rabbits whose prothrombin was reduced to less than 10 per cent invariably caused death due to hemopericardium This indicated a loss of the organism's ability to form a thrombus to seal the mural puncture Specific experimental proof that throm-

bus formation is inhibited by dicumarol was furnished by Dale and Jaques (28) who used the technique already referred to under the section of heparin (7) Richards and Cortell (81) likewise found that dicumarol reduced the tendency of thrombosis following the injection of ethanolamine oleate into the peripheral veins of dogs Bollman and Preston (11) have also noted the ability of dicumarol to prevent intravascular clotting and have utilized this finding for preparing animals to be used in physiological experiments that require non-coagulable blood

Essentially the inhibition of platelet agglutination is the primary step in the prevention of thrombosis When the prothrombin is reduced to a low level, and the coagulation is greatly delayed, platelet clumping no longer occurs as Dale and Jaques (28) have convincingly demonstrated Baronofsky and Quick (5) found that no agglutination in vitro of rabbit blood occurred after the coagulation time was prolonged by dicumarol, and that an accurate platelet count could be made without using any additional anticoagulant Obviously one of the essential needs for the rational use of dicumarol clinically is an exact study correlating the coagulation time and the level of prothrombin with the inhibition of platelet agglutination

*Salicylates as the cause of hypoprothrombinemia* Link and his associates (59) noted that rats on a vitamin K poor diet when given salicylates either orally or intravenously developed a hypoprothrombinemia which could be completely prevented or counteracted by giving the animal synthetic vitamin K Dogs and rabbits were found to be much more resistant to the action of salicylates, but after liver injury with chloroform a reduction of prothrombin could be effected with this drug Rapoport and his associates (79) independently also discovered this action of salicylates and showed that not only animals, but also children could develop a low prothrombin due to large doses of sodium salicylate or acetylsalicylic acid Meyer and Howard (68) observed that relatively small doses of salicylates produced demonstrable hypoprothrombinemia, and in agreement with Link found that vitamin K prevented this effect Further confirmatory evidence concerning the decrease of prothrombin from salicylates has come from Shapiro and his group (95) They record the interesting finding that dicumarol and salicylates complement each other's action In cirrhosis the action of salicylates is more pronounced It seems that the consensus of opinion of all these workers is that the action of the salicylates is essentially the same as that of dicumarol but is less powerful

Little work has been done on the correlation of structure and the physiological action of drugs depressing prothrombin activity Fantl (34) has found that 3 methyl 4-hydroxy coumarin and methylene-bis-(-dimethyldihydroxyresorcinol) is inactive, but that the homolog of dicumarol 3,3′ ethylidene bis (4-hydroxy-coumarin) has the property of producing hypoprothrombinemia which is however less severe and shorter in duration

The action of sulfaguanidine in reducing the prothrombin level of the blood presents a number of important aspects (10) Vitamin K restores the prothrombin concentration but has no effect in reversing the growth inhibition, *p*-amino-

benzoic acid as well as liver extract restores both growth and the prothrombin level The most obvious explanation is that sulfaguanidine inhibits bacterial activity on which the rat depends for at least part of its vitamin K requirement Since *p*-áminobenzoic acid is also effective when given parenterally, the simple explanation does not appear adequate as pointed out by the authors Collins and Hoffmann (23) report a hemorrhagic diathesis in a case of sprue in which succinylsulfathiazol produced a severe hypoprothrombinemia Recently Wakim, and his associates (110) demonstrated that cecectomized rats on a vitamin K-free diet when given succinylsulfathiazol invariably developed a severe hypoprothrombinemia, which in many terminated with hemorrhage Normal rats on the same diet and with the same quantity of the drug only occasionally showed a reduction of prothrombin Obviously the sulfonamides *per se* have no demonstrable action on prothrombin, but the members of the group which specifically act against intestinal infection can cause a lowered prothrombin, probably by suppressing bacterial activity and thereby reducing the synthesis of vitamin K The prothrombinopenia is therefore due to lack of this vitamin

## SUMMARY

The normal antithrombin of the blood is closely associated with the albumin fraction It does not inhibit or retard coagulation, but merely inactivates thrombin Quantitative methods have been developed for its determination, but the significance of its variation in the blood remains obscure

Heparin, the natural physiological anticoagulant, is a compound closely related to mucoitin polysulfuric acid Due to its strongly acidic character, it forms complexes with various proteins and other biological compounds Heparin *per se* is not an antithrombin, but with a co-factor present in serum albumin forms a strong thrombin-inactivating complex Heparin in inhibiting the conversion of prothrombin to thrombin likewise requires a co-factor which is present in the plasma Heparin prevents the agglutination of platelets, probably by virtue of its anticoagulant action The function of heparin in the body has not been determined It is liberated during anaphylactic and peptone shock, but the mechanism whereby this is brought about is still obscure and the purpose of this physiological response has not been ascertained

Antithromboplastin has been demonstrated in the blood, and evidence has been found that it is abnormally increased in hemophilic blood

Dicumarol is the toxic principle isolated from spoiled sweet clover hay It is a coumarin derivative which had not hitherto been known to occur in plants When dicumarol is administered orally or intravenously to man or animals, it causes a gradual but severe decrease of the prothrombin of the blood (or more accurately of component B of prothrombin) Several days are required for the production of its full effect, and recovery is equally slow Evidence is accumulating which suggests that vitamin K has some antagonistic action against dicumarol The hypoprothrombinemia produced by the drug can be temporarily alleviated by transfusion Dicumarol appears to have no toxic action other than depressing the prothrombin except in excessive dosage It is probable that some

of the pathological findings in fatal cases of dicumarol poisoning can be attributed to tissue anoxia due to the severe anemia after excessive hemorrhage

Salicylates especially when given to animals on a low vitamin K diet depress the prothrombin of the blood but to a much smaller degree than dicumarol Sulfaguanidine and succinylsulfathiazol also cause a hypoprothrombinemia, but the action appears to be due to a depression of the synthesis of vitamin K by the bacteria of the intestines and not to a direct action on the synthesis of prothrombin

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# THE HYPERPNEA OF MUSCULAR EXERCISE

JULIUS H COMROE, Jr.

*Department of Pharmacology University of Pennsylvania*

The history of respiratory physiology for the last 60 years has been a succession of attempts to explain all deviations from normal respiration by one theory, whether it be regulation by oxygen content of the arterial blood (133), by alveolar or arterial $CO_2$ tension (71), by arterial blood pH (161), (79) or by acidity of the interior of the cells of the respiratory center itself (61) But no one theory yet offered can account for all or even a large part of the hyperpnea of muscular exercise, and each of these theories has failed to gain general acceptance because of its inability to explain this most common and most powerful of all respiratory adjustments Of one thing, however, there can now be little doubt, namely, that the action of known chemical substances directly upon the respiratory center can no longer be regarded as the most important feature in the control of respiration (141) This seemingly drastic conclusion has resulted from several factors, one of which was the brilliant discovery of the pressure receptors in the carotid sinus and aortic arch and of the chemoreceptors in the carotid and aortic bodies (83) This led to the realization that since reflexes had been demonstrated clearly to assume a share of respiratory control formerly accredited to the action of chemical substances upon the respiratory center, then other anomalies of respiration previously classified as 'exceptions to the rule' might also be due to reflex factors As a result of the new outlook, sufficient experimental work has accumulated in the past decade to warrant an even more revolutionary statement Control of respiration by $CO_2$ appears to be limited to man at rest or performing mild exercise (and to such laboratory experiments as voluntary hyperventilation and inhalation of $CO_2$ mixtures), under all other conditions, increased ventilation is caused by factors other than $CO_2$, and while these may be varied, usually they are of reflex origin An inquiry into the causes of the hyperpnea of muscular exercise will document this statement for this hyperpnea entails the interaction of practically all the factors known to influence respiration, both central and reflex

This review will attempt to evaluate the importance of each of these factors and to estimate the contribution of each to the total hyperpnea of exertion

1 The effect upon respiration of metabolites formed during exercise A *The action of $CO_2$* "If ever there was a conviction firmly entrenched in physiology, it was the monopoly of the chemical control of breathing by the respiratory center Based as it was upon circumstantial evidence, it proved to be one of physiology's outstanding creeds Central stimulation was simply taken for granted Indirect evidence was accepted as direct proof Statements going unchallenged were eventually accepted as facts' (62)

This creed was firmly established by the brilliant work of Haldane (72) who demonstrated beyond doubt that $CO_2$ is a powerful respiratory stimulant and that the normal respiratory center is exquisitely sensitive to changes in alveolar

or arterial $CO_2$ tension This has been amply confirmed by many investigators Campbell, Douglas and Hobson (31) showed that a 2 mm increase in alveolar $CO_2$ tension led to a ventilation increase of 10 liters per min, Douglas and Havard (54) demonstrated an increased respiration of 32 liters per minute with an increase of only 5 6 mm in alveolar $CO_2$ tension, Barcroft and Margaria (7) obtained a maximum ventilation of 71 liters per minute upon inhalation of 7 5 per cent $CO_2$

It was only logical to consider that the hyperpnea of exercise might be due to the increased $CO_2$ produced by the actively working muscles with consequent stimulation of the respiratory center Indeed Haldane found an increase in alveolar $pCO_2$ in mild exertion and this has been confirmed by Hough (90) However an overwhelming amount of evidence now exists to indicate that alveolar or arterial $pCO_2$ does not play an important rôle For example, Barcroft and Margaria (7) showed that while severe muscular exercise increased ventilation to as much as 115 liters per minute, the maximum hyperpnea obtainable with inhaled $CO_2$ in the same subject was only 71 liters per minute, this has been confirmed by Nielsen (119) and indicates that some factor or factors in addition to $CO_2$ must operate to produce a ventilation almost double that possible with $CO_2$

Furthermore quite early it became manifest that there was no positive correlation between alveolar or arterial $pCO_2$ and ventilation during exercise Hough (90) showed that while alveolar $pCO_2$ was elevated during mild exercise, it usually returned to normal with more severe exercise This has been confirmed by Harrison and co-workers (75) and by Krogh and Lindhard (99) In severe muscular exertion, the alveolar and arterial $pCO_2$ are invariably reduced below normal by 1 to 13 5 mm (90, 103, 50, 25, 45, 14, 41) It must be concluded *a*, that under no circumstances can the maximal hyperpnea of exertion be due to $CO_2$ alone, and *b*, since the $pCO_2$ is rarely above normal in moderate exercise and never above normal in strenuous exertion, the rôle of $CO_2$ must be a minor one limited to mild muscular movements However, the marked increase in metabolic $CO_2$ does prevent too great a fall in arterial $pCO_2$ and thus provides a favorable environment for the optimal functioning of the respiratory center, this appears to be a matter of great importance and will be alluded to later

B *The action of lactic acid* Noting the lack of correlation between ventilation and alveolar $pCO_2$, Winterstein (161) and Hasselbalch (79) proposed a modification to Haldane's original concept to the effect that arterial pH rather than $pCO_2$ was the effective regulator of respiration Campbell et al (30, 31) calculated that the respiratory center was so exquisitely sensitive to arterial pH that it responded to changes beyond the limits of existing methods for the detection of pH, a 100 per cent increase in respiration volume was postulated for each decrease in pH of 0 013 unit Despite the fact that respiration was shown to increase in the face of decreased arterial acidity (61) as in anoxemia (27), hemorrhage (5), anemia (13), fever (70, 68), cardiac decompensation (58), intravenous bicarbonate injection (36), and despite Scott's demonstration of a very low sensi-

tivity of the respiratory center pH changes (144), the Winterstein-Hasselbalch hypothesis was accepted by most physiologists It appeared to offer the best explanation for the hyperpnea of muscular exercise for, following Ryffel's discovery of the formation of lactic acid by contracting muscles and its frequent spill-over into the blood stream (134), it was logical to assume that the increased acidity of the blood could provide the stimulus to the center when the arterial $pCO_2$ had fallen below normal There is ample confirmation of the fact that high levels of blood lactate[1] may be found following severe exercise (85) and that blood pH may be reduced markedly (103, 155, 16) Lactic acid is formed when there is a disproportion between the intensity of the muscular work and the oxygen supply to the muscles, it is formed therefore not only during severe muscular exercise in a normal individual but also when milder muscular work is performed during arterial occlusion or during systemic anoxia Static effort, in which the circulation to the active muscles may not increase in proportion to the effort involved, probably represents another example of anaerobic work, though this is disputed by Rein and Talbott (131)

However, it has been demonstrated by a number of investigators (42, 111, 112 118, 40, 123, 25, 73) that blood lactate does not rise during mild exercise as believed formerly (85, 106), and does not necessarily rise with moderate exercise though oxygen consumption or respiration is increased 3–4 fold An initial increase in blood lactate of exercising athletes was found by Bang (6) this was not sustained and returned to a normal level though the work and the hyperpnea continued unabated The severity and duration of exercise required for spill over of lactic acid into the blood varies with the individual (probably depending in part upon training) but at any rate respiration may be increased markedly during exercise with no significant deviations from the normal arterial pH or $pCO_2$ (Harrison et al 75, Nielsen, 120) Bock et al (25) found that De Mar (the famous marathon runner) on one occasion had a ventilation of 90 7 liters per minute with only an insignificant change in lactate and a decrease in arterial $pCO_2$ In addition there is considerable evidence that, when lactate does increase in the blood and pH decreases, there is little or no correlation between minute volume of respiration and arterial pH It has been shown that immediately after severe exercise blood lactate may be increasing (85) and blood pH decreasing (16) while respiration is decreasing abruptly Barr (16) measured arterial pH and respiratory minute volume during and immediately after severe exercise In one instance pH was 7 13 during exercise when ventilation was 60 liters per minute, while after exercise the arterial blood was even more acid (pH 7 09) though ventilation had fallen to 31 7 liters per minute In a second subject, the arterial pH during and after exercise was practically the same (7 20, 7.21) while the corresponding volumes of respiration were 51 and 14 6 liters

[1] High blood lactate does not necessarily indicate a decrease in blood pH since lactic acid reacts with $BHCO_3$ in the blood to form B lactate with the liberation of $CO_2$ (Blood proteins also release some base thus aiding in buffering the lactic acid (155) ) On the other hand a decreased blood pH due to exercise is usually associated with a high level of blood lactate though unidentified acids could be present

Barman et al (9) have presented curves for blood lactate and ventilation in the post exercise period showing poor correlation between the two, lactate remaining high at a time when respiration had returned toward normal Bock et al (25) observed in two exercising subjects who had approximately the same ventilation (45 and 48 liters per minute) that the decreases in pH were markedly different (0 02 and 0 15 respectively)

It seems to be well established therefore that the hyperpnea of muscular exercise is not necessarily due to a decrease in arterial pH It is a much more difficult matter to assess the proportion of the total hyperpnea which may be attributed to changes in arterial blood acidity when the latter occur This question can be approached best by observing the degree of hyperpnea that occurs when similar changes in pH occur in the absence of muscular activity Unfortunately very few measurements have been made of respiratory minute volume in clinical conditions associated with a fall in pH, such as diabetic or nephritic acidosis, and those results that have been recorded are not consistent, cases of equal severity have been reported with pulmonary ventilations as low as 10 6 (113) and 11 4 (98), and as high as 55 (114) liters per min This suggests that many complicating factors, such as fever, heart failure, pneumonia or peripheral circulatory failure, may be present in very ill patients and so prevent a clean cut analysis Consequently investigators have produced a fall in arterial pH experimentally by the ingestion of ammonium chloride in resting healthy men and have come to the conclusion that the respiratory mechanism is relatively insensitive to pH changes Harrison et al found an increase of only 0 1 liter per minute associated with a pH drop of 0 07, Nielsen (120) found a rise of 0 7 liter per minute associated with a fall in pH of 0 08 unit, and Dennig et al (49) observed an increase of 3 1 liters per minute with a drop of 0 2 pH unit

Results such as these have led some to doubt that acidity, either of the arterial blood or of the cells of the respiratory center, is the characteristic stimulus to respiration as suggested by Gesell (61) There is now available acceptable evidence to show that the true stimulus is not acidity but $CO_2$ as originally proposed by Haldane and Priestley in 1905 (71) While it was thought for many years that $CO_2$ acted only by reason of its acidity (its special virtue being the rapidity with which it diffuses through tissues (92)) it now seems more likely that it acts specifically The evidence is as follows Hooker, Wilson and Connett (88) perfused the medullary centers of dogs with two types of blood, one made acid by the addition of 5 per cent $CO_2$ and the other made equally acid by the addition of HCl, marked increases in respiratory volume, as much as 3069 per cent, following perfusion of the $CO_2$-blood while the maximum increase following perfusion of the HCl-blood of the same pH was 128 per cent Since it may be objected that a certain level of $CO_2$ is necessary for the initiation of respiratory movements, and that the $pCO_2$ must have been very low in the HCl-blood, these experiments cannot be regarded as conclusive However more recently evidence of a different type but leading to the same conclusion has been obtained Nielsen (120) working with a normal human subject obtained an increase in respiration of 10 liters per minute by $CO_2$ inhalation with a fall in arterial pH of 0 03

unit, while respiration increased only 0 7 liter per minute following long continued ammonium chloride ingestion with a pH drop of more than twice this magnitude (0 08 unit) In this connection the experiments of Comroe are of interest, he injected solutions directly into the medulla of cats with a microtechnique utilizing the Horsley Clarke apparatus and demonstrated that the cells of the respiratory center frequently could be stimulated by bicarbonate buffers but rarely by injections of lactic or hydrochloric acids (38)

While these experiments indicate that $CO_2$ and not acidity is the natural stimulus to the respiratory center, they obviously do not mean that increased acidity does not stimulate respiration at all Regardless of the controversial question as to whether acidity constitutes a strong, moderate or weak stimulus at the center it can stimulate respiration powerfully through reflexes aroused in the carotid bodies (see p 327) A compromise view which is in agreement with the few observations reported, would be that small changes in acidity stimulate only weakly while large changes (0.3 to 0 4 unit) may produce powerful respiratory stimulation Many more well controlled observations of respiratory minute volume and arterial pH (over the whole range compatible with life) should be obtained on otherwise normal subjects in order to settle this point In view of the deeply rooted conviction among physiologists that acidity and respiration are closely correlated, it is amazing to see how few measurements have actually been made

C *Effect of other metabolites* There remains the possibility that products of muscle metabolism other than $CO_2$ or lactic acid may be responsible for the hyperpnea of muscular exercise, such as the unidentified chemical stimulant postulated by Geppert and Zuntz (60) or the ' respiratory X of Y Henderson (67) It is improbable that lactate has any specific functions as a respiratory stimulant (38) There is some doubt as to whether potassium normally enters the blood from exercising muscles (96) but it definitely diffuses into the venous blood when the arterial circulation is occluded (2) it could stimulate respiration centrally or reflexly through the carotid and aortic bodies but it is apt to depress in slightly higher concentrations (57, 38 89, 160) Phosphates, formerly thought to increase in the blood during muscular exercise (123), probably do not (59) Other products of muscle metabolism have not been tested for their possible effects upon the respiratory center

2 Reflexes arising from the exercising limbs Two factors delayed recognition of the part played by reflexes from the exercising limbs one was complete satisfaction with the Haldane concept and the other was the fact that Geppert and Zuntz (60) in 1888 felt that they had excluded satisfactorily any reflex factor when they stimulated the lower limbs electrically in dogs and obtained respiratory stimulation after transection of the spinal cord[3] Consequently when Harrison et al (74, 75, 76) described reflexes from moving limbs their

[3] It is possible that Geppert and Zuntz tetanized the limb muscles if such were the case they were dealing not with rhythmic movement of the limbs but rather with static effort which tends (by its limitation of blood flow through the muscles) to produce anaerobic contraction and lactic acid formation

work went practically unnoticed In five dogs with the leg amputated at the hip (the femur fixed in a vise) leaving only the femoral vessels and sciatic nerve intact, passive movements at the rate of 300 per minute led to an immediate augmentation of respiration To be sure the results were not striking, the increases in minute volume of respiration in these experiments being 5 6, 5 9, 3 5, 12 and 32 6 per cent when the vessels were open and 3 3, 6 0, 1 0, 12 0 and 21 per cent when the vessels were occluded, after section of the sciatic nerves, no increase in ventilation was obtained However, this work has been confirmed and extended recently by Comroe and Schmidt (37) In their experiments, passive movements (200/min ) of disarticulated legs in anesthetized dogs increased respiration from 22 to 125 per cent (average, 52 per cent) In 86 tests in 50 normal human subjects, passive movements of one leg at the knee at the rate of 100 per minute led to an average increase in pulmonary ventilation of 40 per cent The hyperpnea could be abolished by nerve section or by local anesthesia about the knee in dogs and by spinal anesthesia in man It did not appear to arise from the stretch of muscle or muscle tendons and, by exclusion, the site of origin was assigned to joint receptors

Though it appears certain that some type of proprioreceptors can set up reflexes from an exercised limb, more precise information is needed as to the contribution of these reflexes to the total hyperpnea of exertion Comroe and Schmidt felt that these reflexes were not powerful enough to account for more than a small share of the hyperpnea of exercise, furthermore the available data leave no provision for adjustment of pulmonary ventilation to the load placed on the muscles, but only to the rate and extent to which the limbs are moved A recent comparison in 27 experiments upon 16 normal men of the effect upon respiration of active versus passive movements (of the same rate and extent) of one leg at the knee has shown the following while active movements resulted in a 57 per cent increase in minute volume during the first minute, passive movements resulted in only a 34 per cent increase, with active movements respiration increased further in the second minute (to 70 per cent), while with passive movements it declined to only 20 per cent (39) In this mild type of exercise, passive movements accounted for an average of 60 per cent of the total hyperpnea However the response was not well sustained, due either to rapid adaptation of the receptors or to the decrease in arterial $pCO_2$ produced by the hyperpnea, this indicates that this reflex drive must be a weak one, for strong reflex hyperpneas such as that of anoxia are well maintained despite a fall in arterial $pCO_2$ It is possible that if a large number of joints (ankle, knee, hip, elbow, shoulder) were brought into play at once, bilaterally, as in severe exercise, this reflex might assume more importance, especially if the arterial $pCO_2$ were maintained by concomitant active contractions of muscles The experiments of Barman, Moreira and Consolazio (11, 10), purporting to show that there is little or no reflex drive during exercise, actually do just the reverse and provide experimental data minimizing the rôle played by chemical substances from the limbs In their experiments, subjects walked on a treadmill until a steady state was reached, the average pulmonary ventilation in 11 experiments on three subjects

was 41.3 liters per minute at this time While the subjects were still walking at the same rate, arterial and venous circulation to both legs was occluded for 1½ minutes by cuffs around the upper thighs, during this period ventilation decreased to 38 6 liters per minute, a fall of only 2 7 liters per minute or 6.5 per cent of the steady state ventilation Since respiration falls off by 40 to 55 per cent in an average individual in the first 1½ minutes of rest after exercise of similar severity (142 7, 135, 101, 124) it would seem that the difference between 40 to 55 per cent and 6 5 per cent (33.5 to 49 5 per cent) could represent the contribution of reflexes from the moving limbs (or of other factors such as discomfort or pain) Further experiments should be done along this line (Barman pointed to the intense hyperpnea occurring after release of the occluding cuffs as important evidence that the hyperpnea of muscular exercise is due predominantly to central stimulation by liberated metabolites It must be emphasized that these conditions are far from physiological and give no information as to the situation existing normally During complete occlusion $CO_2$ lactic acid and potassium may accumulate in the tissues and be swept into the re-established circulation, changes in venous pressure certainly and arterial pressure possibly also occur along with changes in blood temperature Occlusion of the vessels during exercise cannot be counted upon with certainty to do more than establish or rule out the existence of a reflex component )

Experiments employing electrical stimulation have shown that a marked increase in ventilation may be produced by excitation of certain sensory nerves in the limbs Krogh and Lindhard (100), using the Bergonié apparatus for inducing muscular contraction in man by electrical stimuli, succeeded in producing hyperpnea without pain or discomfort, without a rise in alveolar $pCO_2$ and obviously without irradiation from the motor cortex For corresponding quantities of oxygen absorbed, electrically induced work produced a greater hyperventilation than voluntary work in one experiment ventilation increased to more than 70 liters per minute with an oxygen absorption of only 800 cc per minute This would indicate the existence of peripheral nerves or receptors capable of stimulating respiration when these are excited electrically As a rule, however, Krogh and Lindhard found that the electrical stimulation was associated with pain or discomfort Y Henderson (81) found that marked hyperpnea occurred in dogs with mechanical or electrical stimulation of the central end of the cut sciatic nerve, he attributed this to stimulation of pain fibers Faradic stimulation of both legs in human subjects increased respiration from 6.58 to 13 95 liters per minute when only moderate discomfort was experienced, when the stimulus was increased, respiration increased to 25 6 liters per minute but intense pain was perceived (20) Since pain is not ordinarily encountered in dynamic muscular exercise, these receptors would appear to be of little importance However in static effort (34) (such as holding a weight or supporting oneself on parallel bars) discomfort or even pain may be a prominent feature (20) For example the simple extension of one leg in mid air led to an increase in ventilation to 34 7 liters per minute at the end of the fourth minute, the subject had to discontinue the effort because of the intense discomfort (20) This may be due

to the fact that muscle spasm limits the blood flow through the muscle and thus favors the production of lactic acid and the sensation of fatigue (though the former has been doubted by Rein (131) and the latter by Jervell (93)).

3. CHEMICAL RECEPTORS IN THE LIMBS. This raises the very interesting question as to whether there might be receptors in the limb which respond to concentrations of muscle metabolites lower than those which produce pain. It is known that chemical factors formed during muscular exercise may produce vasodilatation, pain (105), and a reflex increase in blood pressure (1); it is also known that chemoreceptors capable of stimulating respiration exist elsewhere in the body (carotid bodies (83), aortic bodies (140), lower respiratory tract (136)). The possibility of the existence of similar receptors in the limbs has been investigated in a variety of ways. Comroe and Schmidt (37) were unable to demonstrate reflex stimulation of respiration in man, cat, or dog by total ischemia or by products of muscle metabolism formed by exercise during complete ischemia; if pain was avoided exercise during ischemia produced no greater hyperpnea than exercise alone. The latter has been confirmed by Barman et al. (11); these authors, however, found a measurable increase in respiration during ischemia alone. Delucchi (48) found that the respiratory increase produced by static effort of the legs was increased when cuffs around the thighs were inflated to a pressure two-thirds that of the systolic blood pressure; since no discomfort was experienced, this could be regarded as evidence for the existence of chemoreceptors in the limbs. However the respiratory changes were very small (11–16 per cent) and at no time was the leg circulation completely occluded.

A large variety of substances including creatine, phosphocreatine, blood from exercised ischemic muscles, anoxic blood, hypercapnic blood and acidic blood, was injected or perfused intra-arterially into the limb muscles of cats and dogs without the production of any reflex stimulation of respiration (37). Moore et al. found that acids (pH 6.0 or less) and alkalies (pH 9.2 or more) injected intra-arterially in cats often produced transient reflex respiratory stimulation (116). Neutral solutions of salts produced similar hyperpnea if their concentration was great enough. Of the chlorides tested barium, rubidium and potassium stimulated in lowest concentrations; of the sodium salts tested citrate, phosphate, fluoride and lactate stimulated in lowest concentrations (the threshold for stimulation with neutral sodium lactate was 0.17 M concentration). Moore et al. have shown satisfactorily that this reflex arises in the smaller vessels of the limb and that it probably represents a response to pain. It cannot be of any physiological significance in ordinary types of exercise though it is conceivable that metabolites formed within the muscle cells might stimulate receptors there without the production of pain.

4. REFLEXES FROM THE CAROTID AND AORTIC BODIES. The characteristic stimulus to these chemoreceptors is a decreased oxygen tension of the arterial blood, though unusual changes in $pCO_2$ or pH may also lead to reflex alteration of respiration (140). While relative anoxia may exist in the exercising muscles in exhausting exertion, a drop in arterial blood $pO_2$ rarely occurs in healthy individuals. Himwich and Barr found that arterial oxygen content and saturation

increased in subjects performing brief periods of vigorous work (86), similar observations have been recorded by other investigators (20, 24, 87 89) The fact that inhalation of oxygen may lead to less dyspnea and to greater capacity for exertion (82, 121) indicates that the increase in total oxygen carried by the blood (which amounts to 10 per cent during inhalation of 100 per cent oxygen) reduces the degree of local oxygen lack in the muscles, it does not indicate a reduction of carotid and aortic body activity for inhalation of 100 per cent oxygen usually leads to a slight increase in respiration in subjects with 95 to 96 per cent saturation before the inhalation (146 159)

It is true however that arterial oxygen saturation may fall rarely following exhausting activity in normal subjects (77) and frequently following even moderate exercise in patients with cardiac disease anemia, emphysema, tuberculosis pulmonary fibrosis and polycythemia vera (87, 95, 78) the magnitude of the fall varies as a rule from 2 to 10 per cent, though in two patients it amounted to 21 per cent and 55 per cent In general however, arterial anoxemia is not a factor in the hyperpnea of exercise, the increased alveolar $pO_2$ resulting from the increased depth of respiration appears to be sufficient to maintain or increase oxygenation even in the face of a greatly increased pulmonary circulation rate and a decreased $pO_2$ in the venous blood

So far as other stimuli to these chemoreceptors are concerned the arterial $pCO_2$ never rises high enough under physiological conditions to reach the threshold for carotid body stimulation, consequently any effects attributed to $CO_2$ must be due to action upon the respiratory center (139, 140)

So far as the response of the chemoreceptors to changes in pH is concerned it was pointed out earlier (see p 323) that respiration may be stimulated reflexly by acidity The only experiments reported in which pH changes were produced without concomitant $pCO_2$ changes were saline perfusions of the carotid bodies, in 12 dogs it was found that a decrease of 0 1 pH unit increased ventilation 10 to 50 per cent (7 observations), 0 2 unit 10 to 50 per cent (6 observations), 0.3 unit 0 to 200 per cent (0 observations) and 0 4 unit 20 to 300 per cent (15 observations (139)) Since pH changes of 0 1 have been observed frequently during exercise (16) and changes as great as 0 4 unit have been reported occasionally (103 155), it is possible that these chemoreceptors may account for all or a large share of the hyperpnea of exercise due to increased arterial blood acidity The above experiments were not technically perfect (phosphate buffers were used to alter the pH of Locke solutions in which both calcium and potassium chlorides were present precipitation of calcium phosphate may have occurred thus leaving a preponderance of potassium ions which in themselves could stimulate the carotid bodies) they should be repeated and further attempts made to determine the sensitivity of the deafferented respiratory center to pH changes (the experiments of Hooker et al (88) were performed before the discovery of the carotid bodies)

Reflex stimulation of respiration by warming the chemoreceptors was demonstrated by Bernthal and Weeks (23) who showed a 32 6 per cent increase in respiration upon changing the temperature of the carotid body from 39° to

43°C., this was not a panting type of respiration since both rate and depth were increased. This was confirmed by Schmidt, Comroe and Dripps who obtained an increase of 70 per cent in minute volume when the temperature of fluid perfusing the carotid bodies was raised from 38 to 41°C. The relation of this hyperpnea to the increased body temperature observed in muscular exercise will be discussed later (see p. 329).

5. Reflexes from the lungs. Since clinical dyspnea, such as that occurring in congestive heart failure, is thought to be due in large part to an increased amount of blood in the lungs (55, 46, 35), it is conceivable that this factor may be involved in the hyperpnea of severe exertion. While there is no doubt that blood is flowing more rapidly through the pulmonary vessels in view of the marked increase in cardiac output in exercise, this does not necessarily mean that there is an increased quantity of blood in the lungs. The total amount of blood in the lungs has been estimated by vital capacity determinations in man before and immediately after exercise. A decrease in vital capacity has been found occasionally, chiefly in long races of the marathon type; in a group of 20 such runners, vital capacity was 17 per cent lower at the end of the race, but the men were so fatigued that this change was due probably to physical inability to blow into the apparatus (63). The majority of investigators have found that only insignificant changes occur in vital capacity following short but strenuous exercise. Harrison (75) found changes ranging from −150 to +200 cc. in 8 individuals (average change was −6 cc.). Iglauer and Altschule found changes varying from −230 cc. to +70 cc. in 15 healthy adults; in 12 subjects the vital capacity decreased and the average change in the 15 subjects was −80 cc. (91). Similar results were reported by Levine and Wilson (104). These slight changes are within the limits of error of the method.

It is possible that a very slight decrease in vital capacity may be sufficient to set up powerful respiratory reflexes and that the method may not be sensitive enough to detect such small changes. To answer this, we can examine the relation between vital capacity and pulmonary ventilation in cardiac decompensation, for it is generally admitted that here the increased amount of blood in the lungs is a major factor in producing dyspnea. Actually we find that very gross decreases may occur in vital capacity without correspondingly great increases in the volume of respiration. Campbell found that cardiacs with dyspnea had an average vital capacity of 53.5 per cent and pulmonary ventilation of 7.2 liters per minute; normal subjects with an average vital capacity of 95 per cent had ventilation amounting to 6.9 liters per minute (32). Peabody and Sturgis averaged respiration in 11 normals (vital capacity 101 per cent) and found a volume of 7.16 liters per minute; in 11 cardiacs (vital capacity 83.6 per cent) the volume was only 9.12 liters per minute (126). Barr and Peters reported similar results (129), respiratory volume being 10.8 liters per minute in four cardiac patients with moderate dyspnea as opposed to 6.6 liters in normal subjects (12). It is probable therefore that the very small changes in vital capacity detected in muscular exercise must have little or no effect upon respiration. It must always be borne in mind however that even small reflex stimuli may produce

greater effects when the arterial $pCO_2$ is maintained (152, 99) as it is in muscular exercise by increased formation of $CO_2$, in congestive heart failure, the arterial $pCO_2$ is reduced (130) (128) by the reflex drive and not maintained by excess $CO_2$ production On the other hand reduction in vital capacity, whether due to increased blood in lungs or to pneumothorax or to constriction of the chest by a tight swathe (153), increases ventilation entirely by an increase in rate, the depth being reduced, this rapid shallow respiration is not characteristic of the hyperpnea of exercise, in which both depth and rate are increased

Another possibility to consider is the actual formation of edema fluid in the alveoli concomitant with a higher pulmonary blood pressure in exercise, this may be the cause of the decreased vital capacity noted at the conclusion of marathon races and of the persistent cough occasionally experienced after gruelling exercise though Gordon et al were unable to hear rales in the chest at such times (63) It would be interesting to obtain figures for arterial oxygen saturation in these conditions

6 Reflexes from the great veins and auricle Bainbridge has described a cardio-accelerator reflex arising from the great veins and auricle in response to increased pressure in those structures (3) Since this reflex is an important factor in producing the acceleration of the pulse in exercise, it might conceivably operate also toward increasing respiration Harrison (75) felt that an increase in pressure in the great veins and right auricle increased respiration by reflexes carried over the vagi but his experimental procedures are all open to question he increased the venous pressure by rapid infusions of large amounts of fluids into the external jugular vein and by distention of the right auricle with a balloon Respiratory volume was increased consistently by 50–100 per cent but it is obvious that too many variables existed in such experiments to permit final conclusions

Evidence that venous pressure does rise in muscular exercise was obtained by Schneider and Collins who found that venous pressure rose as the intensity of the work increased (143) With 4000 ft lbs per minute the venous pressure averaged 30 per cent above normal at 6000 it was 50 per cent and at 8000 it was 75 to 95 per cent above normal The drop in venous pressure after the cessation of exercise followed the usual curve for the return of ventilation to normal—i e, it dropped rapidly in the first and second minutes after exercise and then more slowly, reaching the normal level in 6 to 9 minutes (though with very severe work full recovery was delayed 22 to 27 min ) Obviously the stimulus exists in the great veins during muscular exercise if it can be shown that increased pressure within the vein (without interfering with blood flow) actually does stimulate respiration reflexly

7 Effect of increase in body temperature upon respiration It is known that severe muscular exercise may result in an increase in body temperature but the extent to which body temperature may rise and its effect upon pulmonary ventilation have been generally disregarded Many investigators have reported moderate increases in body temperature during exercise (8, 21, 22, 29, 42, 108, 109, 122, 127) and Hill and Flack have reported an instance in

which the temperature rose to 40 5°C after severe exercise However there have been very few measurements of the effect of an increase in body temperature upon respiratory volume The older literature contains only scattered and incomplete observations of the effect of induced temperature increase upon the respiration of resting subjects Graham and Poulton (64) and Haldane (70) noted dyspnea when the rectal temperature exceeded 38 9°C, at 40 2°C the respirations were 44 per minute (64) Sutton noted an increase in respiratory rate of 5–6 per minute for every degree C rise in rectal temperature, "the depth and energy" of respiration increased also (154) A decrease in alveolar $pCO_2$ during fever was noted by Haggard (68) and by Bazett and Haldane (17) However the first quantitative studies were those performed by Landis et al (102), by the use of hot baths, body temperature was raised to varying heights In one subject, when the rectal temperature reached 40 3°C, the ventilation was 34 4 liters per minute, at 39 7°C, the ventilation was 21 6 liters per minute, and yet the alveolar $pCO_2$ had fallen in these experiments to 15 6–22 6 mm If increase in body temperature in a resting subject can increase respiration to 34 4 liters per minute in spite of a great reduction in alveolar $pCO_2$, it is reasonable to suppose that an even greater hyperpnea might occur if the alveolar $pCO_2$ were maintained nearer normal as is the case in exercise Probably even less striking increases in temperature could contribute a large share of the total hyperpnea when the temperature rise is due to exercise

On the basis of the available evidence, the following evaluation may be made of the contribution of the temperature rise to the total hyperpnea of exercise The temperature rise is certainly not responsible for the onset of the hyperventilation for the onset of the former is too slow The few continuous measurements of rectal temperature made during exercise show that temperature may rise within 1 to 2 minutes, certainly within five minutes (22, 21, 122) Since, in steady state exercise, the hyperpnea reaches its maximum before the temperature reaches its peak, the temperature rise cannot be largely responsible for the hyperpnea Furthermore the temperature rise varies in different individuals performing the same amount of work (84) Yet it is difficult to escape the conclusion that fever must be of great importance in explaining the hyperventilation that occurs in very strenuous exertion when the body temperature rises dramatically It is also likely that the temperature increase can explain the long recovery period following muscular exercise for the body temperature and respiration return to normal at about the same time (42), (21), except for the first three minutes after exercise (when the respiratory volume falls rapidly), the temperature and respiration curves are parallel

Increased body temperature may increase respiration in a number of ways *a*, via the carotid and aortic bodies (23, 139), *b*, via the cerebral centers cephalad to the medulla (see Bazett (19)), *c*, via increase in metabolism of the medullary respiratory centers (145, 18), *d*, by decreasing blood pH (149, 150), by the action of increased temperature on blood as a physicochemical system, and *e*, by peripheral reflex factors There is least support for the last of these mechanisms though Nasset and Peters (117) found that a greater increase in respiration fol-

lowed heating both head and trunk (separated vascularly) than either alone, and Boycott and Haldane (27) reported that "warmth causes a slight fall in alveolar $pCO_2$ without any rise in internal (rectal) temperature"

8 EFFECT OF ADRENALIN LIBERATION The injection of adrenalin may lead to depression (apnea) or stimulation of respiration or to both depending upon the circumstances and mode of administration Following intravenous injection of adrenalin with an increase in blood pressure McDowall (110) observed an apnea (now known to be due largely to pressorreceptor reflexes (83)) followed by a more lasting stimulation of respiration Griffith et al found that adrenalin increased respiratory volume 15 to 28 per cent in chloralosed cats (65) and Schmidt (138) noted that adrenalin is an excellent respiratory stimulant in cases of depressed respiration Starr and co-workers (151) reported that 0 67 cc adrenalin given subcutaneously to normal human subjects increased minute volume of respiration 42 per cent (this representing a considerably greater effect than that produced by the so-called respiratory stimulants such as caffeine theophylline and strychnine) This increase in ventilation following adrenalin may be due to the increased metabolism that it is known to produce It may also be due to an increase in blood lactate, Courtice Douglas and Priestley (43) noted that blood lactate rose from a normal of 10 mgm per 100 cc to 33–37 following adrenalin injection and similar figures have been reported by Cook and Hurst (40) In addition to these two actions, adrenalin may have a specific stimulant effect upon the central nervous system of the type seen with other sympathomimetic amines It is unlikely that the stimulant effect observed by Starr is due to an improvement in cerebral blood flow for ephedrine raised blood pressure more but produced less respiratory stimulation furthermore an increase in cerebral blood flow would tend to decrease respiration by reducing the amount of stimulant material within the cells of the respiratory center (61) (137)

This increase in respiration following adrenalin suggests another mechanism by which respiration might be increased even further in severe exercise There appears to be little doubt that adrenalin liberation does occur during muscular movements in cats (33) The extent to which such liberation occurs in man has not yet been agreed upon (52) Cannon's method for determining adrenalin liberation in cats by using the rate of the denervated heart as an indicator might conceivably be applied to man in view of the recent increase in sympathetic surgery recently There is little disagreement, though, that adrenalin does enter the circulation in exhausting exercise

9 EFFECT OF IRRADIATION FROM THE MOTOR CORTEX Krogh's hypothesis that impulses from the motor cortex radiate to the respiratory center and increase its sensitivity has not yet been tested experimentally, evidence in favor of this theory was obtained chiefly by exclusion or lack of other factors known at that time Krogh and Lindhard (99) felt that this concept was necessary in order to explain the immediate increase in respiration that occurs within the first second after beginning exercise, that in addition it was necessary to explain the increased respiration in spite of a decreased arterial $pCO_2$ However the need for this hypothesis has now largely disappeared The immediate increase in respiration

at the start of exercise may be explained on a reflex basis as demonstrated by the work of Harrison et al (75), Comroe and Schmidt (37) and indeed by the experiments upon electrically induced work performed by Krogh and Lindhard (100), in none of these experiments was the motor cortex involved

Krogh's hypothesis may still be needed to explain the magnitude of the full hyperpnea of exercise but further evaluation of the rôle played by the other factors concerned is needed before this can be settled It is possible that the sum of the effects produced by afferent nerve impulses, pH changes, temperature increase, maintenance of arterial $pCO_2$, venous pressure rise, adrenalin liberation all acting in harmony may provide a complete explanation At any rate further evaluation of known factors and search for new factors susceptible of experimental proof offers a more satisfying approach than acceptance of his hypothesis The only experimental work dealing with the effects of cortical irradiation (electrical stimulation of the motor cortex of animals) upon respiration has not provided positive evidence cortical stimulation produced either no change in respiration or an increase in rate with a decrease in depth (148) Further experiments of this sort are desirable

10 Effect of internal acidity of cells of respiratory center The hypothesis (first emphasized by Gesell (61)) that alterations in respiratory activity were governed not by arterial $pCO_2$ or pH but by the acidity of the cells of the center, was necessitated by the lack of correlation between arterial $pCO_2$ and pH and respiration, and the urge for a unitarian theory of respiratory regulation Since such a theory can never be susceptible of direct proof until methods for measuring the pH of the cells of the respiratory center without injury are devised, its acceptance must depend wholly upon the lack of other satisfactory explanations Such a lack no longer exists the hyperpnea of anoxemia[3], fever, ether inhalation, acidosis, cardiac decompensation, and low blood pressure may now be explained in large part or wholly by new factors, usually reflex and all capable of objective proof Likewise the hyperpnea of muscular exercise no longer requires a theoretical approach Furthermore such a hypothesis never satisfactorily explained the hyperpnea of muscular exercise for the hyperpnea begins simultaneously with the first movements If the center turns more acid in order to produce this initial gasp, why does it do so? It cannot spontaneously increase its own metabolism, there must be some stimulus to the center to produce this initial augmentation of activity whether it be from the motor cortex or reflexly from the limbs, and it is the origin of these stimuli that is our chief concern

## CONCLUSIONS

Among the earliest explanations proposed for the control of respiration was that of Volkmann in 1841 (158) and Vierordt in 1844 (157), who believed that respiration was regulated chiefly by means of reflexes One hundred years later this

[3] Davenport et al (47) observed a delayed increase in rate and minute volume of respiration occurring in unanesthetized dogs (with carotid and aortic bodies denervated) when made to breathe 9 to 18 per cent oxygen, this could be classed as an instance of increased respiration due to increased acidity centrally if all other possibilities are excluded

explanation again seems to be a very satisfactory one, though resting now on far firmer experimental support In the intervening years all theories dealing with the control of respiration were concerned chiefly with the dominant effect of chemical substances acting directly upon the medullary centers (133), (60), (94), (72), (61) Probably the most important discovery of the last century—more important than the finding of any new reflex or chemical stimulant—has been the realization that respiration is controlled not by reflexes alone not by chemical stimulation of the medulla alone, but by the proper interaction of both factors No reflex, no matter how strong, can stimulate respiration if the arterial $CO_2$ tension has been lowered abnormally (99, 152), no chemical stimulant, no matter how great, can produce rhythmic breathing if the medullary centers have been completely cut off from all nervous influences including that residing in the pneumotaxic center (152) Respiratory alterations in general, and the hyperpnea of exercise in particular, cannot be explained by any single simple theory but only by a consideration of a number of known and probably many unidentified factors

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# LACTATION

W E PETERSEN

*University of Minnesota, Minneapolis*

The literature dealing with the various phases of lactation is far more extensive than can be adequately treated in a single review The object in this treating therefore shall not be to consider all of the literature on the subject but rather to review the more recent contributions and refer to older literature only as it is deemed necessary to establish a foundation, or to make contrasts It too shall be the object to make conclusions where the evidence seems to merit them and to present all recent evidence on both sides on controversial questions

The physiological and biochemical aspects of lactation can best be treated by considering in order each of the three distinct phases it involves The first of these is the development of the mammary glands The second phase is the initiation and maintenance of lactation and the third is the evacuation of the milk in the gland While endocrines are prominently involved in all three phases, biochemical aspects are the more prominent in the second phase and need consideration for a proper understanding of the lactation phenomenon

Recent reviews by Turner (1939), Riddle (1940, 1941), Hisaw and Astwood (1942) and Petersen (1942b) deal with the relation of the endocrines to lactation The literature pertaining to the biochemical as well as the endocrine aspects of lactation has been reviewed by Folley (1040a) and Petersen (1942a) and in book form by Espe (1941)

Development of the mammary glands The ovarian hormones are well established as the agents responsible for the mammary growth in normal animals Although there are some exceptions, in general, the estrogens cause duct growth while progesterone brings about development of the lobule-alveolar system when administered to the ovariectomized but otherwise normal animal Controversy exists as to the mode of mediation of mammary growth by these hormones It is also clear that other than ovarian hormones are essential for complete mammary development Evidence has been presented that adrenal, thyroid, and hypophyseal hormones play a part synergistically Placental hormones are also involved The state of nutrition must also be considered as well as the effect of male hormones

*Exceptions to the general concept* That estrogens cause only duct growth does not hold for all species or conditions In the guinea pig Nelson (1936) and others, and in the monkey Allen (1927) and Gardner and Van Wagenen (1938) have shown that complete lobule-alveolar development was obtained with estrogen administration to ovariectomized females In the rat small amounts of alveolar development following estrogens have been observed by Nelson (1935) and others In the mouse Gardner (1935) and in the rabbit Lyons (1936) have made similar observations In the ovariectomized cow, Walker and Stanley (1941) obtained apparently complete mammary development with diethylstilbestrol as did Lewis and Turner (1941a) in an ovariectomized goat

Since diethylstilbestrol was made available a large number of studies on its effect upon mammary development has been reported In most cases this estrogen has been administered to animals with intact ovaries, and, therefore, some of the observed effects may be due to the action of some naturally secreted ovarian hormones It should also be pointed out that in most cases and with larger animals in particular complete mammary development has been assumed when the lactation was copious and not by necropsy or biopsy inspection Large mammary development in goats by administration of diethylstilbestrol has been reported by Lewis and Turner (1942b), Folley and Young (1941a) and others Folley, Scott Watson and Bottomley (1941a b) Reece (1943), Lewis and Turner (1942a), Walker and Stanley (1941) and others have reported upon mammary development and milk flow simulating that expected following a normal parturition to result from diethylstilbestrol administration to nulliparous heifers Petersen and Boyd (1944) have found uniformly good response to stilbestrol administration in the nulliparous heifers but only occasionally did multiparous dry cows respond with well developed mammary glands or high lactation levels. This failure is difficult to explain as in the multiparous animal full mammary development had occurred in a previous lactation and it would seem that a subsequent lobule-alveolar development to stilbestrol should be more readily obtained It is also noteworthy that in 5 freemartins and several nymphomaniacs with chronic ovarian cysts very little mammary development or lactation occurred

In general estrogen administration must be more intensive and prolonged in the male than in the female to obtain the same degree of development and in some cases complete development is not obtainable Lewis and Turner (1941a 1942c) report some lobule-alveolar development in male mice, rats, rabbits and guinea pigs as occurring only after prolonged treatment with large doses. The same workers (1942b) were unable to obtain any significant development in the male goat even after prolonged treatment with large doses Petersen and Boyd (1944) treated a steer and a bull for 5 months with diethylstilbestrol and obtained but slight mammary enlargement and a few milliliters of milk daily Failure to obtain response to estrogen treatment in the freemartin male, and castrate male could be explained by postulating an absence of progesterone in these classes Such postulation would be difficult to reconcile with the results obtained by Walker and Stanley (1941) who obtained full development and lactation in the ovariectomized bovine Walker (192) concluded that progesterone is not needed for complete mammary gland development in the bovine

In the human female stilbestrol has been used successfully by MacBryde (1939) and others to develop hypotrophic breasts Dunn (1941) has reported a case of gynecomastia in the human male as the result of oral administration of stilbestrol

While estrogen may cause various degrees of development of the lobule-alveolar system, Selye (1940a 1940b) found that large doses of progesterone without pretreatment with estrogens produced mammary development comparable to late pregnancy in spayed rats. Similar reports have been made by Selye, Bor

duas and Masson (1942) and Reece and Bivins (1942) for rats and by Hartman and Speert (1941) for monkeys

*Synergism of estrogen and progesterone* The synergistic action of estrogen and progesterone upon the uterus and sexual behavior has been the subject of much study Recently the problem of quantitative relationship of the two hormones in mammary development has been undertaken by Lyons and McGinity (1941), Scharf and Lyons (1941) with rabbits and by Mixner and Turner (1943) with mice These reports indicate that maximal effects are obtained with 1 mgm progesterone to 240 I U estrone

Mixner and Turner (1943) postulated that estrogen acts directly upon the tissues producing hyperemia and greater vascular permeability permitting other hormones to enter the tissues in increasing amounts The vasodilating action of estrogen in the periphery has been shown by Abramson, Zazeela and Schkloven (1941), Hechter, Lev and Soskin (1940) and others A large number of reports on the hyperemic effect of estrogen upon the uterus is to be found Since the mammary gland is an accessory reproductive organ the estrogenic effect upon the uterus is of special interest because similar effects upon it can be expected Among the more recent studies Reynolds (1939, 1941) and Kerly (1940) found that with the hyperemia of the uterus caused by estrogen there was an increase in the tissue water as well as in metabolism Increased permeability of the capillaries of the estrogen treated uterus was demonstrated by Hechter, Krohn, and Harris (1941) by intravenous injection of trypan blue

Direct evidence for the local effect of estrogen is to be found in the several reports on its percutaneous application and observing development only in those glands so treated Among others, such observations have been made on the mouse by Gardner (1941) and Gardner and Chamberlin (1941), on the rat by Lyon and Sako (1940), on the guinea pig by Nelson (1941a) and Jadassohn et al (1937), on the rabbit by Lewis and Turner (1942), on the monkey by Speert (1940, 1941a) and Chamberlin et al (1941), on the goat by Folley et al (1940, 1941c) on woman by MacBryde (1939) and on the cow by the reviewer and Folley et al (1941c)

It therefore appears that one of the main, if not the chief function, of estrogen is to increase the vascular system and its permeability in the mammary gland to progesterone, other hormones and metabolites If sufficiently large concentrations of progesterone are administered enough will get through the capillaries to produce lobule-alveolar growth without preconditioning to estrogen The inhibitory effects of excess doses of estrogen upon mammary growth reported by Gardner (1940a) are difficult to reconcile with these views

*Rôle of the hypophysis* The usual failure of obtaining mammary development by estrogen and progesterone administration in hypophysectomized animals prove without question that pituitary hormones are involved As to the identity of these hormones there is controversy and conflicting evidence One school contends that estrogen acts upon the hypophysis to form a duct growth factor and progesterone acts similarly to produce a lobule-alveolar growth factor Others contend that the ovarian hormones act directly upon the mam-

mary glands but must be assisted by a number of naturally occurring hypophyseal hormones.

Since Corner (1930) first demonstrated that administration of an anterior hypophyseal extract to hypophysectomized animals permitted of mammary development numerous attempts have been made to ascertain the pituitary fractions involved The Missouri group have been the chief proponents of the hypothesis that estrogen and progesterone act upon the hypophysis producing mammogen I and mammogen II respectively that in turn mediate the mammary growth Evidence for support of their hypothesis is presented in a series of reports beginning with Gomez and Turner (1937a) and Gomez, Turner, and Reece (1937), that implantation of pituitaries from rats treated with estrogen caused mammary development in hypophysectomized guinea pigs while pituitary implants from nontreated rats failed Later Gomez and Turner (1938) reported similar results on hypophysectomized rabbits and rats with pituitary implants from pregnant and non pregnant cattle Lewis and Turner (1938, 1939) and Lewis, Turner and Gomez (1939) reported upon the lipoid nature of the duct growth factor (mammogen I) and methods for biological assay In the latter report they extend confirmatory evidence on the identity of the duct growth factor still maintaining the factor is lipoid in nature In the latest report Trentin, Lewis, Bergman and Turner (1943) admit error in the original claim that mammogen I is lipoid and now claim it is protein in nature.

That progesterone acts upon the pituitary o produce a factor (mammogen II) which mediates lobule-alveolar development was proposed by Mixner (1940) Mixner and Turner (1941a) reported on a method for biological assay for the factor by the use of male mice and in 1942a on the rôle of estrogen in the stimulation of the lobule alveolar growth Mixner, Bergman and Turner (1942) present further claims for the specific lobule-alveolar growth factor in the hypophysis as they found no parallelism between the lactogenic, thyrotropic and gonadotropic activities and mammogenic potency The work of Gomez (1942) lends support to the lobule-alveolar factor being a separate entity from other known hypophyseal factors He found that a hot alcohol-ether extract of the fresh hypophysis contained the lobule-alveolar growth principle and none of the other established pituitary hormones Mixner and Turner (1943) report more extensively on studies of the hypophyseal lobule-alveolar factor and suggest it is protein in nature

While there is no question about pituitary hormones being essential for mammary growth there is strong opposition to the hypothesis that special hormones are formed in the hypophysis by the action of the ovarian hormones Hisaw and Astwood (1942) conclude such a hypothesis to be untenable in view of the well established local effect of percutaneous application of estrogen to mammary glands previously cited In further support of this conclusion may be cited the several reports failing to confirm several phases of the reports on mammogen" Reece and Leonard (1939) found no difference in the mammary growth stimulation obtained in hypophysectomized rats in implants of pituitaries from estrogen treated and nontreated animals Nelson (1938, 1939a)

made similar observations The failure of Selye and Collip (1936) to obtain mammary development from pituitary implants is difficult to explain in light of the above results Greep and Stavely (1941) failed to observe any mammogenic effect of a lipoid extract of pituitary glands according to Lewis and Turner (1938) while the lipoid extracted material exerted marked influence on mammary growth The reviewer and co-workers (unpublished) have made similar observations Mixner and Turner (1943) explain the failure of obtaining differences from implants of pituitaries from estrogen and nontreated animals in the hypophysectomized animals as being due to the lack of sensitivity in the operated individuals as compared to intact male mice which they used as test animals

Successes in replacement therapy by various hypophyseal hormones strongly suggest that naturally occurring pituitary hormones act in conjunction with the ovarian hormones to produce mammary development Although not corroborated by Nelson (1938, 1939), Greep and Stavely (1941), Reece and Leonard (1939) and others, the fact that Lewis and Turner (1939) and Mixner and Turner (1943) obtained larger amounts of duct growth and lobule-alveolar growth respectively, from the hypophysis in pregnancy than from that of non-pregnant or male animals it is possible that in pregnancy certain metabolic hormones needed for mammary development are increased in the hypophysis

The development of the mammary gland is a growth phenomenon and would therefore be expected to involve the action of several of the principles of the hypophysis affecting metabolism The general rôle of the hypophysis in this relation has recently been reviewed by Long (1942) Inanition, an invariable sequela to hypophysectomy, has been shown by Astwood, Geschicter and Rausch (1937) to prevent mammary development in otherwise normal rats The work of Nathanson et al (1939) supports, and Trentin and Turner (1941) confirmed, this finding but found that greatly increased doses of estrogen would cause mammary duct proliferation in inanition Although inanition is undoubtedly a factor in the failure of complete mammary development in ovarian hormone administration in hypophysectomized animal it is not the only one as Samuels, Reinecke and Petersen (1941) noted no greater development in hypophysectomized rats that were force fed by stomach tube so as to gain in weight than those not so treated In a more recent report, Samuels, Reineke and Bauman (1943) found that the weight increments in force fed hypophysectomized animals was due to fat storage There was a loss of the nitrogen stores

In view of these facts it is surprising that response in hypophysectomized animals should be obtained with anything less than a therapy of a complete extract of the hypophysis yet there is a growing literature reporting on varying degrees of development obtained by the administration of various hypophyseal fractions in conjunction with ovarian and other hormones Frederickson (1939) obtained mammary development in hypophysectomized rabbits with estrogen and progesterone but was unable to obtain lactation from such glands with prolactin unless the animal was pregnant Gardner (1940) obtained some mammary development in hypophysectomized male mice with desoxycorticosterone, progesterone, and estradiol depropionate Nelson (1941b) reported

that the andrenotropic hypophyseal fraction caused slight to marked mammary development in castrated hypophysectomized rats of both sexes. Chamorro (1940) obtained lobule-alveolar development by administration of desoxycorticosterone acetate and estrogen to hypophysectomized male rats. Gardner and White (1941) obtained mammary development from estrogen and lactogen concluding that estrogen sensitizes the gland to the action of the lactogen. Later Gardner and White (1942) found that when an adrenotropic extract was administered together with estrogen and lactogen still better development was obtained. A saline extract of pituitaries, ineffective by itself, greatly enhanced the action of the estrogen and lactogen. This further emphasizes the probability of estrogen working in conjunction with hypophyseal hormones. That the growth hormone is involved is indicated by a number of reports. Using hypophysectomized animals, Reece and Leonard (1941) found that growth hormone augmented the mammary growths to estrogen. Later (1942) these workers obtained good lobule-alveolar development in hypophysectomized rats by the administration of growth hormone and testosterone propionate. Leonard (1943) found estradiol dipropionate alone, if injections were begun one day after hypophysectomy, would stimulate end buds but was ineffective 7 days after the operation. Samuels, Petersen, Reinecke and Bauman (unpublished data) too have observed marked beneficial effects from the growth hormone but contend other anterior pituitary hormones are also essential for complete development.

Further evidence that the lactogenic hormone plays an important rôle in development in the normal animal is indicated by a number of recent reports. Astwood (1941) reported that lactogen or a hypophyseal hormone closely associated with it, and for which the term luteotrophin is suggested, maintains and regulates the corpus luteum function. That luteotrophin is distinct from the hypophyseal luteinizing hormone seems to be well established by the reports of Astwood (1941), Evans, Simpson, Lyons and Turpeinen (1941) and Evans, Simpson and Lyons (1941). From these works it appears that the luteinizing hormone only promotes growth of the corpus luteum and the luteotrophic hormone causes it to secrete progesterone. Lyons, Simpson and Evans (1941, 1942, 1943) have added confirmatory evidence and by use of highly purified lactogenic hormone have identified it as the luteotrophin, as has Tobin (1942).

Not only are the results of Lyons (1941), using the guinea pig in certain stages as a test animal for the lactogenic hormone, explained by these new discoveries but they add much to the understanding of the mammary development in pregnancy. The corpus luteum becoming persistent (in some species there is exception) is stimulated by the lactogenic hormone to secrete progesterone which in turn acts upon the mammary gland, conditioned by estrogen, to stimulate lobule-alveolar growth. Other hormones from the hypophysis and other endocrine glands also play more or less important rôles.

*Rôle of the placenta.* That the placenta is capable of taking over the mammogenic functions of the hypophysis is evidenced by at least three recent reports. Desclin (1939) hypophysectomized pregnant guinea pigs and found no signs of

atrophy when autopsied 9 to 12 days later while in males where the glands had been developed by estrogen complete atrophy was observed following hypophysectomy Newton and co-workers (1939, 1941) likewise observed normal glands in hypophysectomized pregnant mice In the first report they expressed the fetuses leaving the placentas *in situ* and found no evidence of atrophy whereas when the placentas as well as the young were expressed atrophy of the mammary glands was complete This experiment should rule out the hypophyseal secretion of the fetuses as the causative factor In the latter report some of the hypophysectomized animals parturated normally and the glands were filled with milk but all young died because of lactation failure

*Rôle of the thyroid gland* The reports on the rôle of the thyroid gland in mammary development are conflicting Dragstedt et al (1924) reported complete mammary development in pregnant dogs deprived of their thyroids and parathyroids when tetany was prevented Leonard and Reece (1941) reported thyroidectomy to enhance mammary growth in both normal and castrated female rats with or without estrogen treatment Smithcors and Leonard (1942) observed thyroidectomy of male mice to inhibit mammary growth A lesser effect was noted in castrated males Gardner (1942) reported orally administered desiccated hyroid to stimulate mammary growth in male mice but not in castrated males Mixner and Turner (1942b) noted improved mammary development in thyroidectomized mice when thyroxine was administered with estrogen and progesterone but not with estrogen alone In cattle Spielman, Petersen and Fitch (1944) observed much less mammary development in thyroidectomized cows and heifers than would be expected in normals Petersen, Knodt and Ludwick (1944) have observed that no apparent mammary development is obtainable by stilbestrol administration in thyroidectomized myxedemic heifers but with simultaneous thyroid administration normal response was obtained

These conflicting results cannot be adequately interpreted on the basis of available experimental evidence The complete development observed by Dragstedt et al (1924) in thyroidectomized pregnant dogs might be accounted for by the thyroxine secretion of the developing fetuses Spielman, Petersen and Fitch (1944) observed no mammary development in pregnant myedemic heifers until about midterm The beginning of mammary development roughly corresponded to the disappearance of myxedema both of which were attributed to the secretion of thyroxine by the developing fetus Because of the rôle the thyroid plays in general metabolism and growth it would be reasonable to believe that it plays a part in optimal mammary development

*The rôle of the adrenals* The function of the adrenals in normal mammary development is speculative That the adrenal hormones can effect mammary development is unquestionable, but whether or not they do so in the normal intact pregnant animals is not definitely proven The beneficial effects of hypophyseal adrenotropic fractions upon mammary development in hypophysectomy observed by Nelson (1941), Gardner and White (1942) and others indicate that probably normal function of the adrenals is essential for complete develop-

ıt That desoxycorticosterone is the mammogenic factor of the adrenals been shown by its effects when injected Van Heuverswyn et al (1938), ›ert (1940b) and others obtained full mammary development in guinea pigs l monkey from desoxycorticosterone injections as did Chamorro (1940) h hypophysectomized male rats when also estrogen treated Leonard and ›ce (1942), however, failed to obtain mammary growth with desoxycortico- rone in hypophysectomized rats with or without estrogen That desoxycorti terone has the mammogenic properties of progesterone is established. Mix- and Turner (1942b) found it to be about one-half as potent as progesterone h mice as the test animals and may therefore augment progesterone under ural conditions Interesting from a speculative point of view is the report Beall and Richstein (1938) of finding progesterone and allopregnanolone in adrenal Of speculative interest is also the report of Butcher (1939) that ·enalectomy enhanced the mammary gland development in underfed albino s

*Effect of other steroids* A comparatively large number of steroids have ›ule-alveolar growth stimulating properties A number of studies on the ımmogenic effect of testosterone propionate has been reported recently lley, Guthkelch and Zuckermann (1939) and Van Wagenen and Folley 939) obtained good lobule-alveolar growth in monkeys Reece and Mixner 939) obtained complete lobule-alveolar development in spayed rats and initia n of secretion They also report a 40 per cent increase of the lactogen content the hypophysis as a result of testosterone propionate injections Lacquer 942, 1943) observed lobule-alveolar growth in rats and sufficient lactation for ›ter sucklings to obtain milk Noble (1939) obtained duct and lobule-alveolar ›wth in intact duct and lobule-alveolar growth in intact rats by testosterone opionate injections but only nipple growth resulted when the animals were ariectomized and hypophysectomized Leonard and Reese (1942) likewise led to obtain mammary growth with testosterone propionate in hypophysec mized rats Forbes (1942) studied the age differences in response to testoster- e propionate implantation finding no mammary response in prepubertal noderate response in pubertal and complete response in post pubertal male ıd female rats This age difference in response may account for some of the nflicts in the literature as to observed effects of various treatments

Reese (1941) found that androsterone was ineffective while Mixner and urner (1942c) found testosterone and testosterone propionate ineffective and ›hydroandrosterone effective in producing mammary development in spayed ice

Mixner and Turner (1941b) reported pregneninolone to have lobule-alveolar ımulating effects Selye (1941) reported acetoxy pregneninolone to be only ıghtly less effective than desoxycorticosterone Mixner and Turner (1942c) ›wever, found it to be only about one-fifth as active Using spayed mice as say animals these workers have reported the mammogenic properties in rms of progesterone for the following steroids to be desoxycorticosterone '2, dehydroandrosterone 1/3, acetoxypregnenolone 1/4, methyl testosterone

1/25, and testosterone and testosterone propionate as being ineffective Unless there is a species difference their failure to get mammary growth response from testosterone and testosterone propionate is difficult to explain as all other work indicates that these substances are effective in promoting lobule-alveolar growth

LACTATION With the exception of the last two years when relatively few publications have appeared a large literature has developed on the complex phenomenon of lactation In general the contributions in this field can be rather sharply divided between those that deal with the endocrine and those that deal with the biochemical and other physiological aspects of the problem While the work in recent years has added much for a better understanding of this complex phenomenon much is still obscure and considerable controversy exists on several details Some of the controversy comes from the rather sharp cleavage between those working on the endocrine and those working on the other physiological aspects of the problem Because of this fact and that there is more or less interdependence of all the physiological aspects of the problem it is deemed advisable to forsake the idea of limiting this review to the endocrine phases and include the biochemical and other physiological problems as well Such a plan obviously necessitates the forsaking of some of the details that a more limited approach would permit

For ease in treatment the discussion will be divided into the following four heads A, endocrine factors, B, time when milk is secreted, C, milk and blood equilibrium factors, and D, synthesis of milk

*A Endocrine Factors* It is well known that the mammary gland may be well developed, such as in pseudopregnancy or by the administration of mammogenic principles without lactation occurring This fact is taken as *a priori* that other factors than those necessary for mammary development are essential for lactation In search for these factors studies have led to the possible rôles played by the hypophysis, thyroid, adrenals and pancreas

1 *The anterior pituitary* Stricker and Grueter (1928) started the long list of investigations of the hypophyseal function in lactation when they reported initiation of lactation in pseudopregnant ovariectomized rabbits by the injection of an aqueous extract of the anterior hypophysis Since then efforts have been directed toward the identification of the hypophyseal fractions that are lactogenic While major efforts have been expended in studies on the so-called lactogenic factor (prolactin) conclusive evidence is at hand to indicate other hypophyseal fractions contribute in a major way The reports on effects of various fractions are difficult to evaluate in many instances because of the impurity of the substances used Until very recently none of the so-called AP fractions have been free from contamination and now only one, prolactin, is obtainable, in pure form

Although much of the effect upon lactation attributed to prolactin is due to substances with which the preparation used was contaminated a number of facts point to its being one of the hypophyseal lactation factors While Gardner and Turner (1933) and Nelson (1934) have proposed other methods for analysis the standard test animal is the pigeon and the test is based upon the crop growth

stimulation It is significant that the prolactin content of the hypophysis varies as to species, physiologic stage and age, first reported by Bates et al (1935), and since added to by many Reece and Turner (1937a) found the hypophysis of dairy cattle to contain significantly more of the hormone than those of beef cattle and less in calves than in older animals In another report (1937b) these workers found the administration of estrogen to increase the prolactin content of the hypophysis In another report, Reece and Turner (1937c) found the stimulus of nursing to increase the hormone Reece (1939) working with guinea pigs found the greatest hypophyseal concentration of the hormone in lactation with successively decreasing amounts for late pregnancy, estrus, early pregnancy and diestrus Holst and Turner (1939) found no increase in early pregnancy, little increase in late pregnancy, and large increases following parturition in rabbits and guinea pigs In pseudopregnant rabbits, Meites and Turner (1942a) found no increase in the prolactin to that of the normal Lewis and Turner (1941b) were able markedly to increase the prolactin content of rat pituitaries by the administration of stilbestrol and (1942b) obtained similar results with estrone injections in male rabbits Prolactin has also been identified in the urine of lactating women by Lyons and Page (1941), Turner and Meites (1941) and Ehrhardt and Voller (1939), in the blood and urine of pregnant and lactating mares by Leblond (1937), in placentas by Turner and Meites (1941) and in the urine of babies secreting witch's milk by Lyons (1937a)

The differences observed by Chance et al (1939) in the prolactin content of the hypophysis in different species shows no relation to their lactation capacities They report but little of the hormone in the hypophysis of the horse, most in the sheep, with man, swine and cattle in between in ascending order

Since lactation is abolished by hypophysectomy it is only natural that replacement therapy in the hypophysectomized animal should be used in the earlier work in a study of the lactogenic hormone or hormones of the hypophysis Among the successes in maintaining lactation in the hypophysectomized animals by administration of pituitary extracts are the reports of Riddle et al (1933) for rats, Lyons et al (1933) and Housay (1935a, 1936) for the dog Mephail (1935) for the cat, Stricker and Grueter (1928) for the rabbit Nelson and Gaunt (1936) and Gomez and Turner (1936) found that a more purified preparation of prolactin failed to initiate lactation in hypophysectomized guinea pigs and pointed the way for search of other hypophyseal factors involved in lactation

The effect of anterior pituitary extracts administered to larger normal animals has been reported by a number of workers. Grueter (1930), Evans (1933) and Asdell et al (1936) observed marked stimulation of lactation in goats as did Kabak and Kisilstein (1933) in sheep In the cow Grueter and Stricker (1929), Asimov and Krouze (1937), Folley and Young (1937, 1938, 1939) and others have reported marked increases in milk production subsequent to the injection of a crude extract of the anterior hypophysis

Stockklausner and Daum (1932) are the only ones to have reported a decline in lactation in cows following hypophyseal extract administration In all

experiments of this kind it has been found that lactation increases are the greatest when treatment is made in the declining phase of the lactation It should also be noted that the benefits from treatment are as a rule only temporary even when treatment is continued over a period of time (Folley and Young, 1940, 1941a)

As with hypophysectomized laboratory animals when the more purified prolactin was administered, Folley and Young (1939, 1940, 1941b) and Sykes et al (1942) found the benefits to lactation are greatly diminished The reports of Bergman and Turner (1940) and Lyons (1937b) in which they obtained engorgement of the glands of pseudopregnant rabbits with prolactin treatment may seem confusing However, these had intact hypophyses presumably capable of producing the other necessary hormones There is also a question as to the quantity of milk that can be produced under those conditions and certainly the preparations used were not pure prolactin as now produced by the method of Li et al (1940 to 1941)

From these reports it is obvious that the anterior pituitary contains lactogenic principles and it is also equally clear that prolactin is not the only lactogenic hormone A question may even be raised as to the function of prolactin in lactation as it is not peculiar to the lactating female It is found in pituitaries from all animals and classes Rabald and Voss (1939) have shown it to be present in the liver and Lyons (1937) found it in the urine of men Prolactin in lactation is therefore not a problem of quality but rather one of quantity

The lactogenic hormone has been used extensively in attempts to increase lactation in the puerperium with mostly negative results Kayser (1940), Stewart and Pratt (1939), Werner (1939) and many others were unable to observe any beneficial effects from the administration of relatively large doses and frequently undesirable reactions were noted Kenny and King (1939) and Kurzrok et al (1939) are among the few reporting benefits to lactation in women from lactogen

The effect of prolactin upon carbohydrate metabolism is suggested by Folley and Young (1941b) as possibly being more important to lactation than the direct action upon the mammary gland In a recent report Schooley et al (1941) re-emphasized the rôle of prolactin in enlarging the abdominal viscera, promoting body growth and increasing appetitite in the pigeon The luteotrophic properties of prolactin recently discovered and reviewed in the previous section is also a matter to consider in the re-evaluation of the rôle of prolactin in lactation This is especially so in light of the report by Lyons (1941) where lactation was induced in normal female guinea pigs by injection of prolactin commencing not later than the third day following estrus When the injections were started on the fourth day following estrus, or later, lactation was not initiated These results are difficult to explain as the assumption must be that the action of the prolactin must be through the corpus luteum and there is no evidence that this body is necessary for lactation Ovariectomized animals often have been brought into and maintained in lactation

The adrenotropic hormone has recently been shown to be essential and without it lactation can neither be initiated nor maintained Some of the successes with

so-called prolactin preparations must be laid to the contamination of such preparations with the adrenotropic fraction

2 *The adrenals* It has been known for some time that ablation of the adrenals causes complete inhibition of lactation (Carr, 1931, Swingle and Pfiffner, 1932, Gaunt, 1933, and many others) Administration of cortical extracts prevented lactation failures and before the purification of the various adrenal cortical hormones led to speculation as to the active principle A number of reports including Nelson and Gaunt (1936, 1937a, 1937b) Schultze (1937) and Gomez and Turner (1936, 1937b) have obtained complete relief from adrenalectomy symptoms by administration of extracts of the adrenal cortex This has led to speculation as to the active principle Brownell et al (1933) proposed a special lactation hormone in the adrenals and more recently Spoor, Hartman and Brownell (1941) have reported that an extract from the adrenals, not prolactin, is more powerful in pigeon crop stimulation than the adrenal (cortin) hormone Gaunt and Tobin (1936) and Hurst, Meites and Turner (1942), however, were unable to detect any lactogenic hormones in the cortical extract.

With the advent of the isolation and purification of the different cortical principles new light has been shed on the fractions involved in lactation Gaunt (1941a, 1941b) has shown that desoxycorticosterone is incapable of maintaining lactation after adrenalectomy As a matter of fact, there was evidence that it had an inhibitory effect. Nelson and Gaunt (1937a) however, observed that a little lactation could be induced in hypophysectomized guinea pigs with lactogen and a high salt diet. Again these workers (1937b) found in the rat that desoxycorticosterone had a slightly beneficial effect upon lactation They concluded as did Levenstein (1937) that alterations in the electrolyte and water balance such as are affected by desoxycorticosterone have an adverse effect upon lactation

That carbohydrate metabolism disturbance is more important than the water and electrolyte balance while suggested by Gomez and Turner (1936, 1937b) was demonstrated by Gaunt, Eversole and Kendall (1942) and Nelson, Gaunt and Schweizer (1943) They showed that lactation could not be induced in the hypophysectomized guinea pig with lactogen and desoxycorticosterone but with the carbohydrate metabolism fraction, 17 hydroxy 11-dehydrocorticosterone and lactogen copious lactation was induced They also report that desoxycorticosterone had an inhibitory effect upon lactation in the guinea pig The report of Last (1941) that a crude AP extract plus prolactin caused lactation in adrenalectomized rats is difficult to reconcile.

The influence of the hypophysis upon the adrenal is well known Apparently there is no reciprocal effect, at least insofar as prolactin is concerned Meites, Trentin and Turner (1942) have reported that the lactogen content of the rat hypophysis is not affected in adrenalectomy Jones and Nelson (1942) propose a significant relation between the hypophysis and the adrenals They found that stilbestrol acts upon the hypophysis to stimulate secretion of the adrenotropic hormone and it in turn causes increased secretion of the adrenal carbohydrate metabolism hormone

As in other cases in which endocrines are involved the pregnant state exerts an influence Tobin (1940) found that adrenalectomy before or during pregnancy does not necessarily cause abortion but lactation failed after parturition even when the glands were well developed

Since progesterone can substitute for desoxycorticosterone to some extent maintenance of the corpus luteum during pregnancy may be a factor in the well being of the pregnant adrenalectomized animal The effect of secretions by the placenta and fetus, however, should not be overlooked

On the basis of the work reported to date a conclusion is warranted that the adrenals serve in lactation primarily by the secretion of the carbohydrate metabolism hormone 17-hydroxy-11-dehydrocorticosterone Also that desoxycorticosterone is necessary to prevent water and electrolyte disturbances but in larger quantities has an inhibitory effect upon lactation

3 *The thyroid* The relation of the thyroid to lactation continues to be the subject of investigation by means of thyroidectomy, hypophysectomy, and thyroid administration to normal lactating animals Considerable disagreement exists as to the rôle of this gland in lactation Dragstedt et al (1924) found that thyroidectomy did not influence lactation in the dog provided tetany was prevented Nelson and Tobin (1937), Karnofsky (1942) and Nelson (1939b) concluded similarly for the rat Folley (1938, 1942), Prenheim (1940) and Folley, Scott Watson and Amoroso (1942), however, report significant diminution in milk secretion following thyroidectomy in this species In the goat Trautmann (1919) reported marked decreases in thyroidectomized animals while Hibbs et al (1941) obtained lactation for more than a year following thyroidectomy In the thyroidectomized cow, Graham (1934a) reported only a slight depression in milk yield while Spielman et al (1944) noted sharp reduction in milk production

In thyroidectomized animals no evidence exists that the administration of either thyroxine or the thyrotropic hormone assists in the initiation of lactation

In the administration of thyroid and thyroxine to intact lactating animals the large number of reports warrants the conclusion that such administration will increase milk yields and fat percentage Graham (1934a, 1934b), Folley and White (1936), Herman et al (1938), Hurst et al (1940), Dastur and Smith (1939), Ralston et al (1940), and Reinecke and Turner (1942) have reported increases in both milk yield and fat percentage following thyroid or thyroxine administration to cows Jack and Bechdel (1935) observed only increased milk yield De Fremery (1936) observed a decrease in milk yield in goats as a result of thyroxine injections probably due to too large doses Probably for the same reason Grumbrecht and Von Düsterlo (1937) obtained decreased lactation in the guinea pig following injection of the thyrotropic hormone While Bergman and Turner (1943) could not demonstrate any increase in the thyrotropic hormone in the hypophysis of pregnant and lactating rabbits, in contrast Folley and Young (1939, 1940) found hypohyseal extracts containing the thyrotropic principle more effective in increasing milk yields than preparations without this factor

It is apparent that the thyroid is not essential for the initiation of lactation and therefore is not lactogenic in the strict sense That the thyroid hormone exerts a significant influence upon lactation must also be concluded. Its influence on lactation is undoubtedly due to its effects upon metabolism Since milk fat yields are affected more than total milk the report of Dastur and Smith (1939) that thyroxine administration causes a decrease in phospholipids, glycerides and fatty acids in the blood are of interest In these animals the milk fat was increased by 40 to 50 per cent

Dinitrophenol, while having marked effects upon the metabolic rate was found by Graf et al (1940) to have no effect on milk yields in cows when administered in small doses With large doses there was a marked diminution of milk yields as well as marked changes in milk composition Brower and Martin (1938) made similar observations on goats

While as early as 1924 Dragstedt et al reported that it was necessary to control tetany resulting from ablation of the parathyroids in order to maintain the level of milk yield in the dog it remained for Folley (1941a) and Folley, Scott Watson and Amoroso (1942) to demonstrate that the parathyroids are essential for optimum milk yields

4 *Rôle of pancreas* No recent reports on the relation of the pancreas has been found The work of Nelson, Chaikoff and Lyons (1933) indicates that some lactation may take place in the depancreatized dog although it is greatly impaired At this time there is no evidence that insulin has any direct action upon the mammary gland but that its effect upon carbohydrate metabolism is responsible for the influence on lactation

5 *Starting lactation* In normal conditions the mammary gland develops during pregnancy, and lactation as it is usually understood begins following parturition Much evidence *vida supra* has been presented that the lactogenic hormone is greatly increased at the time lactation begins As to the mechanism responsible for the release of the lactogenic hormones at this time little concrete evidence and much speculation has been advanced These speculations fall into 4 main groups as brought out by Meites and Turner (1942c) as follows 1, corpus luteum, 2, the placenta, 3, mechanical distention of the uterus and 4, the estrogen

One of the oldest postulates is that the corpus luteum prevents onset of lactation Expression of the corpus luteum in goats by Drummond Robinson and Asdell (1926) and in rats by Selye et al (1934) resulted in lactation with the obvious conclusion that this body was the inhibitory factor Anselmino and Hoffman (1936) and Folley and Kon (1937) and others have failed to note any inhibition of lactation from the injection of progesterone into lactating animals indicating that if the corpus luteum is the responsible body the inhibition must be due to some other secretion than the progestational hormone. The hypothesis, however, is untenable in the light of the well known fact that copious lactation may prevail during pregnancy with well developed corpora lutea in woman, the cow, and the goat

That the placenta inhibits lactation is supported by the work of Frankl

(1923), Nelson (1934) and Smith and Smith (1933) who observed inhibition of lactation when placentas were retained or transplanted In contrast, however, Litt (1933) with rabbits and Selye et al (1934) with mice were unable to observe any inhibitory effect upon implantation of placentas Further evidence against this hypothesis is the fact that placentas which are frequently retained in cows do not inhibit lactation and also the well known fact that in several species copious lactation may occur during the entire pregnancy

Mechanical distention of the uterus as the inhibitory factor is subject to the same objections as a satisfactory explanation of the problem Selye (1934) and Selye et al (1934) found filling the uterus with parafin following removal of the young by Caesarian section prevented lactation Bradbury (1941) and Greene (1941) using the same technique could not confirm this observation They explained the apparent failure to be due to the effects of the operation as, following recovery, from the effects of the operation they observed lactation in animals with their uteri filled with paraffin

That estrogen will inhibit lactation in animals is well established by a large number of reports but much larger doses are needed for this effect than would be present in pregnancy Among the many reports dealing with the experimental inhibition of lactation are those of De Jongh (1933) and Robson (1935) for the mouse, Reece and Turner (1937), Bacsich and Folley (1939) and Folley and Kon (1937) for the rat, and Folley (1936, 1941c) for the cow

Among the large number who have reported success in inhibiting lactation and preventing painful post-partum engorgement of the breasts are Kurzrok, Birnberg and Livingston (1942), Jeppson, Kasabch and Kanter (1942), Diddle, Nagyfy and Sells (1942), Diddle and Keettel (1942) and Barnes (1942) The reports of Arabanel and Goodfriend (1941) and Arabanel and Klein (1941) confirm the effects of estrogen on preventing painful postpartum engorgement of the breasts but they question the inhibitory effect upon lactation claiming that lactation will continue if the infants nurse Novak (1943) on the basis of clinical experience concurs with this view

Those who have observed inhibitory effects of estrogen upon lactation found that treatment to be effective must begin in the early puerperium When lactation had become established no inhibitory effects were noted Administration of diethylstilbestrol to lactating cows by Folley et al (1941d) and Spielman et al (1941) caused no diminution in milk yields but enriched the milk in fat, protein and sugar The androgens, particularly testosterone, have also been used clinically to inhibit lactation and to relieve painful post-partum engorgement of the breasts with varied success Jeppson et al (1942) reported testosterone propionate was helpful in inhibiting lactation but of no value in relieving painful engorged breasts in the puerperium Fleischner and Kushner (1941), however, reported that testosterone offered complete relief of pain and engorgement in 68 per cent of their patients and Hellman and Auer (1941) found it to inhibit lactation in 35 out of 77 women

Nelson, Gaunt and Schweizer (1943), Gaunt, Eversole and Nelson (1942) and others have observed that desoxycorticosterone has an inhibitory effect upon

lactation and that guinea pigs are more sensitive than are rats Since estrogen testosterone and desoxycorticosterone all stimulate mammary growth and all have been reported to inhibit lactation, Nelson, Gaunt and Schweizer (1943) advance the idea that all substances that stimulate mammary growth have an inhibitory effect upon lactation The fact that large doses of estrogen failed to inhibit lactation in milking cows, that there is a question about estrogen and testosterone inhibiting lactation in women and that progesterone which stimulates lobule-alveolar lactation growth raises a question about the validity of such a statement under all conditions

The fact that the inhibitory effect of estrogen upon lactation in women has been reported to be far more effective in the early puerperium than when lactation becomes established points to a greater sensitivity of the mammary gland before initiation of lactation to the inhibitory substances. However, prepartum inhibition of lactation is not the only factor involved It is well known that cows may be brought into full lactation before parturition by the stimulus of milking To the reviewer it seems more logical to look for some stimulating factors as being responsible for postpartum lactation than to attempt explaining the phenomenon by the removal of prepartum inhibitory factors

Turner and Meites (1942c) proposed the two primary requisitions for copious lactation as being (a) well developed mammary gland and (b) high lactogen content of the anterior hypophysis Both of these are satisfied before parturition Lyons (1937b) and Bergman and Turner (1940) have shown that milk secretion begins some time before parturition as the enlargement of the mammary gland in late pregnancy is due to the engorgement of the alveoli by secretory products The characteristics of colostrum milk add further support to a prepartum secretion because its chemical and physical properties are those expected as the result of equilibria forces exerted upon retained milk (Petersen and Rigor, 1932a) In view of these facts it is apparent that postpartum milk flow is not due to an initiation of secretion at that time but rather a beginning of ejection of the alveolar contents Ely and Petersen (1941) advanced the hypothesis that ejection of milk from the alveoli was due to oxytocin secreted reflexly by the posterior pituitary as a result of the milking stimulus Since oxytocin is secreted during parturition this will account for the initiation of milk flow which is kept up by the subsequent stimuli of nursing or milking Reece and Turner (1936, 1937c) observed that the act of nursing maintained high levels of lactogen in the pituitary, and Hooker and Williams (1940) reported that lactogen injections retarded the rate of mammary involution Stewart and Pratt (1941) for women and Williams (1941) for mice have added additional evidence for the stimulating effects of nursing upon lactation Other facts supporting the hypothesis that postpartum lactation is due to the introduction of stimulating factors rather than the abolition of inhibitory factors will be dealt with in the section on "let down of milk."

The question may be raised as to how nursing stimulates the production of lactogen in the pituitary suggested by Meites and Turner (1942c) While they suggested a direct effect, that is the nursing stimulus, reflexly stimulated the

secretion, two other alternatives exist Since nursing stimulates the liberation of a posterior pituitary hormone it in turn may stimulate secretion of the lactogenic hormone Another alternative is that evacuation of the mammary gland may be the stimulating factor

B *Time of Milk Secretion* Since no recent reports have appeared on the subject it must be taken for granted that the problem of the time when milk is secreted during the interim between milkings is settled The older view advanced by Isaachsen (1923), Maxwell and Rothera (1915) and more recently by Ingelbrecht (1935) that a large portion of the milk is secreted during the nursing act is untenable The view advanced by Petersen, Palmer and Eckles (1929a), Swett, Miller and Graves (1932), Gowen and Tobbey (1928), that the milk is secreted in the interim between milkings, now prevails The study of pressure relations to the rate of milk secretion by Petersen and Rigor (1932b) and Garrison and Turner (1936) indicates that the rate of milk secretion decreases as the milk accumulates in the alveoli The work of Shaw and Petersen (1940) shows that there is no uptake of blood precursors for milk during the milking and indicates a complete cessation of milk secretion at this time The phenomenon of let down of milk in many cases has been mistaken for milk secretion

C *Equilibrium between Blood and Milk* It is well known that milk and blood have the same total osmotic pressure 6 6 atmospheres What is not generally appreciated is that the two are not in equilibrium and it may well be that the lactogenic hormones have something to do with the establishment of the forces necessary to maintain a state of non-equilibrium between them As yet Simms (1931) is the only one to have studied the problem of differences between the constituents of blood and milk as related to equilibria phenomena According to him, on a molar basis the ratios of concentration in milk as compared to blood are fat, 20, sugar, 40, potassium, 7, calcium, 14, magnesium, 4, and $PO_4$, 7 to 1 Blood on the other hand contains 2, 4 and 8 times as much protein, chlorine and sodium, respectively, as does milk It is significant that none of the major constituents in milk has the same concentration as a similar constituent in the blood

For other constituents the concentrations in the blood and milk are identical This has been shown by Peskett (1934) to be the case with urea and probably the same with uric acid, creatine and creatinine Kolda (1926) reviewed an extensive literature on the passage of blood constituents of a dietary origin into the milk and Petersen and Brereton (1942) have reported on several inhaled substances passing into the milk The mammary gland therefore acts as a permeable membrane to many blood substances while for others there is selectivity One can only speculate on the nature of the selective mechanism The fact that sodium bicarbonate is not found in milk can be accounted for by postulating that the mammary membranes are impermeable to it Postulation of partial permeability will account for those substances found in lesser concentration in milk than in blood Wright (1928) has presented evidence that calcium levels may be built up in the milk by combining with casein and phosphorus and thus maintain a Donnan equilibrium insofar as this constituent is concerned Other

constituents not in equilibrium cannot be explained on this basis and what is more must await further work before a satisfactory explanation can be advanced

The best evidence for the existence of forces that prevent milk from coming into equilibrium with blood is that several conditions will set into motion mutual exchanges between blood and milk constituents Conditions that will bring this about are infusion of various substances into the lactating gland, development of intra-alveolar pressures of sufficient magnitude and by general physiological disturbance including gland pathology

Petersen and Rigor (1932c) and Garrison and Turner (1936) have studied in detail the effects of irrigating the cow's mammary gland with distilled water and various concentrations of solutes Filling the gland immediately after milking with distilled water reduced subsequent milk flow but slightly and had little effect upon the character of the milk. With increasing salt or sugar concentrations in the infused fluid there were progressive decreases in the amounts of secretion and alterations in character In general the values for protein, total solids, mineral matter, catalase and pH increased and lactose decreased The reviewer and co-workers have accumulated considerable unpublished data on the effects of blood serum, gums, gelatin, starch, fats, oils, various other agents thought to have therapeutic value in the treatment of mastitis and have found that only water or water with sulfanilamide have little or no disturbing effect upon either the quantity or quality of milk. Hucker and Lee (1932) reported a low sodium chloride (0 12 per cent) solution as well tolerated While it is strange that an isotonic salt solution should have such a depressing effect upon lactation when infused, stranger still is the marked deleterious effect of infusing back into the gland the freshly drawn milk as reported by Jackson and Rothera (1914), Davidson (1926) and Garrison and Turner (1936) The disturbance by reintroduction of the milk was of a similar magnitude to that of physiological saline solution

The early attempts by Neusch (1910), Isaachsen (1923) and Tgetgel (1926) in determining the secretion pressure developed in the udders of cows by means of manometers connected with the gland sinus were in error They either measured the hydrostatic pressure of the milk in the sinus or the much greater pressure from the let down of the milk Petersen and Rigor (1932b) and Garrison and Turner (1936) attempted to measure the maximum intra-alveolar pressure against which milk would be secreted by insufflating the udder with air by the former, and oxygen by the latter The former found 25 mm Hg and the latter 40 mm Hg as the maximum pressures against which milk will be secreted It is also established that as the intra-alveolar pressures increased the rate of milk secretion decreased The increased milk production observed by dairymen by more frequent milking is explained by such a procedure keeping the intra alveolar pressure to a minimum and getting the optimum rate of milk secretion Ludwick, Spielman and Petersen (1941) milked one side of the udder three times daily to gain 16 per cent in milk production over the side milked but twice daily

The effect of leaving the milk in the mammary gland for varying periods of time after the maximum intra alveolar pressures had developed was found by these workers to alter the composition of the milk Apparently as soon

as milk secretion ceases because of pressure the milk begins to come into equilibrium with the blood Colostrum like milk can be produced at any time of the lactation by suspending milking for a long enough period of time Undoubtedly natural colostrum is the result of filling the alveoli with secretion products some time before parturition so that partial equilibrium with the blood has been established Petersen and Rigor (1932a) showed that once the milk had been changed by refraining from milking, several days of regular milking were required before the milk became restored to normal Porcher and Muffet (1930) observed that the casein decreased and the globulin increased in milk retained in the gland beyond the normal length of time If milking is delayed sufficiently long all of the milk in the gland is resorbed and involution occurs (Wayne, Eckles and Petersen, 1933)

Graf, Ludwick and Petersen (1940) and Brower and Martin (1938) administered dinitrophenol to cows and goats, respectively, and found a marked disturbance in the gland resulting in it becoming permeable to sodium bicarbonate and the milk became alkaline Other changes in the direction of colostrum milk were also observed Apparently this drug had a direct effect upon the permeability of the gland cells Changes in the composition of milk in chronic mastitis are also in the same direction to indicate that any disturbance to the secretory cells affect their permeability to various milk and blood substances

*D The Synthesis of Milk* If the number of contributions to the literature is a criterion the study of the synthesis of milk has suffered more than its share of loss due to the war because very few reports on the subject have appeared during the past two years Immediately preceding this period, by the same criterion, there was great activity in this field One is impressed with the efficiency of the mammary gland Graham, Houchin, Peterson and Turner (1938) calculated that only 10 per cent of the total energy uptake as determined by analysis of incoming and outgoing blood in the goat was used by the gland itself Gaines (1928) calculated the overall efficiency of lactation to be 52 6 per cent in the cow or that 52 6 per cent of the energy intake above maintenance requirements is returnable in the milk

Several people have studied the ratio of blood flow through the gland to the amount of milk secreted Determining the mammary gland uptake of glucose in goats by arterio-venous difference and assuming quantitative conversion of glucose into lactose, Graham, Jones and Kay (1936) calculated 500, and Lintzel (1934) 256 volumes of blood per volume of milk Using the thermostromuhr, Graham (1937b) found a ration of about 250 to 1 which is in agreement with the values obtained earlier by Jung (1933) using a stromuhr Shaw and Petersen (1938c) calculated a ratio in the cow of 387 to 1 on the basis of calcium uptake and 391 to 1 on the combined glucose and lactic acid uptake assuming the latter two are quantitatively used for the formation of lactose

The lack of agreement among the reports cannot be fully explained Part may be due to a state of excitement in the animal when samples are taken or measurements made Shaw and Petersen (1939) observed as much as 14 per cent change in blood concentration due to excitement when blood samples are

taken Reinecke, Stonecipher and Turner (1941) recognizing the effects of excitement have proposed anesthetizing the animal when observations are made but in so doing probably introduce other errors such as changed circulation and metabolism Since there is a question about the disposition of glucose in the mammary gland calculations based upon its quantitative conversion into lactose are subject to question

*Fat metabolism in the mammary gland* Milk fat is quantitatively the most variable constituent in milk Because of its great variation in milk from the same individual it is suggested that its synthesis in the mammary gland is governed by some factors not involved in the secretion of other milk constituents The problem of fat synthesis in the mammary gland has been attacked by many workers and from a number of different angles The first attempt at determining the blood precursors of milk fat was made by Meigs et al (1919) using the Kaufmann and Magne (1906) technique in which they concluded that the blood phospholipids were used for the formation of milk fat Using the arterio-venous difference technique, Blackwood (1934) Lintzel (1934) Graham, Jones and Kay (1936) Maynard, McCay Ellis and Hodson (1938) Shaw and Petersen (1940) and Voris et al (1940) demonstrated that the mammary gland takes up neutral fat from the blood These workers as well as Aten and Hevesy (1938) were unable to detect any uptake of phospholipids

Hilditch and Paul (1936) and Hilditch and Jones (1936) suggested that the short chained fatty acids result from a breakdown of the long chained acids taken up from the blood The work of Shaw and Petersen (1940) demonstrating that more than enough neutral fat is taken up by the mammary gland to account for the milk fat supports this view Additional support to the theory that the short chained acids of milk fat result from partial oxidation and reduction from longer fatty acids is found in the reports of Gowen and Tobbey (1928) and Petersen, Palmer and Eckles (1932b) who found the fat of a lactating mammary gland to be intermediate in composition between milk fat and body fat while that of the non lactating gland was similar to body fat The identification of lipase by Kelly (1938, 1943) and of free fatty acids in the basal part of the secretory-epithelium by Kelly and Petersen (1939) indicated that neutral fats are hydrolyzed in the gland

Because they observed the respiratory quotient of the lactating gland to be greater than unity, Graham, Peterson and Houchin (1938) and Reinecke et al. (1941) concluded that fat must be formed from carbohydrate Shaw (1939) also observed the R Q of the mammary gland is greater than unity The reviewer and co-workers have extensive unpublished data obtained from both the intact animals and from excised glands perfused according to the technique of Petersen, Shaw and Visscher (1941) that also show a respiratory quotient above unity While the classical interpretation is that R Q's above unity indicate fat formation from carbohydrate this is not necessarily so, as pointed out by Soskin (1941)

The recent studies showing that the lactating mammary gland uses $\beta$-hydroxybutyric acid adds speculation as to its function in milk fat synthesis Shaw and

Knodt (1941a) observed that β-hydroxybutyric acid is constantly used by the mammary gland in normal conditions and Shaw (1942) reported more than twice as great usage in a cow suffering from ketosis He calculated that all of the oxygen uptake by the gland would be required for the oxidation of the β-hydroxybutyric acid taken up by the gland in ketosis and about 37 per cent in normal animals In another report Shaw and Knodt (1941b) calculated that the β-hydroxybutyric acid taken up by the lactating mammary gland is sufficient to account for all short chained fatty acids in the milk up to $C_{14}$ More recently Shaw and Petersen (1943) studied the utilization of ketone bodies using the perfusion technique It was found that the β-hydroxybutyric acid utilization increases with increased amounts in the blood and aceto-acetic acid is not used

On the basis of the evidence at hand it is certain that blood phospholipids are not the precursors of milk fat In spite of the contrary indications in the observed R Q it is probable that there is no conversion of carbohydrate to fat in the mammary gland because more than enough neutral fat is taken up to account for the milk fat The part that β-hydroxybutyric acid plays in the fat metabolism of the mammary gland is only speculative at this time

Carbohydrate metabolism in the mammary gland Among the many that have shown that the mammary gland takes up glucose from the blood are Blackwood and Stirling (1932), Lintzel (1934), Graham et al (1936, 1936, 1938) and Shaw, Boyd and Petersen (1938) The assumption is that the blood sugar is used for lactose formation That lactic acid may also be a blood precursor for lactose is indicated by the reports of Graham (1937) and Shaw, Boyd and Petersen (1938) who showed that lactic acid is also removed from the blood These workers calculated that the lactic acid plus glucose removed from the blood would be about enough to account for the lactose in the milk Powell and Shaw (1942) later questioned that lactic acid is removed from the blood under normal conditions as they could not detect any uptake unless cows were excited Reinecke, Williams and Turner (1941) reported that appreciable glycoproteins were removed from the blood and suggest the sugar is split off to become available for the carbohydrate metabolism in the mammary gland

To what extent lactic acid and the sugar part of glycoprotein are involved in lactose synthesis is not known but that glucose is the chief precursor of lactose is obvious Lowering of the blood sugar by insulin administration is reported by Petersen, Hewitt, Boyd and Brown (1931), Brown, Petersen and Gortner (1936b), Gowen and Tobbey (1931b) and others to cause a decrease in the lactose content of the milk Hypoglycemia produced by the administration of phloridzin has been reported by Patton and Cathcart (1911) and Gowen and Tobbey (1931) to produce similar results Similarly reduction in the lactose content of milk was observed by Gowen and Tobbey (1931a) and Overman and Wright (1927) in the hypoglycemia produced in inanition Hyperglycemia is reported to increase the lactose content of the milk when produced by intravenous injections of glucose by Nitzescu (1925) but not by Brown, Petersen and Gortner (1936c) or Petersen and Boyd (1937) Whitnah, Riddell and Hodgson (1933) increased the milk sugar by the introduction of large amounts of sugar into the

stomach of cows by stomach tube Both hyperglycemia and increased lactose in the milk were obtained by Jones (1935) through thyroxine administration and by Bottomley, Folley, Walker and Scott Watson (1939) by subcutaneous implantation of epinephrin tablets Intramammary duct injections of glucose by Brown, Petersen and Gortner (1936c) produced hyperglycemia but no increase in lactose content of the milk in the glands not injected Although the blood sugar was not determined the administration of stilbestrol to lactating cows was found to increase the lactose content of milk by Spielman, Ludwick and Petersen (1941) and Folley, Scott Watson and Bottomley (1941d)

While it is obvious the blood glucose is the chief precursor of milk sugar the mode of its conversion is little understood Grant (1935) obtained lactose synthesis by incubating mammary tissue slices with glucose and with glucose and galactose (1936) Petersen and Shaw (1937) could not demonstrate lactose synthesis from glucose and mammary gland tissue unless lactic acid was added Since the mammary gland contains 0.2 per cent glycogen (Petersen and Shaw, 1938) it would seem that it must play a part in lactose synthesis Whatever the intermediate steps are can only be conjectured That the phosphorylated breakdown products of glycogen occurring in muscles are not involved is indicated by Grant (1936) who could demonstrate no lactose formation by tissue slices when hexose monophosphate or a phosphoglycerate was added

That the systemic carbohydrate metabolism is altered during lactation is shown by Cahane (1938) when following removal of the mammary glands of guinea pigs he noted increases in blood glucose and liver glycogen and a decrease in muscle glycogen His postulation that the lactogen hormone causes a conversion of glycogen to glucose is significant in light of one of the modes of action of lactation hormones

*Nitrogen Metabolism in the Mammary Gland* Very little as yet is known about the nitrogen metabolism in the mammary gland The conclusion by Cary (1920) supported by Blackwood (1932) that blood amino acids are the precursors of milk proteins has proven to be at least partly wrong Graham, Houchin and Turner (1938), Graham, Peterson, Houchin and Turner (1938), Shaw and Petersen (1938a, 1938b) and Reinecke, Peterson, Houchin and Turner (1939) agree that there is uptake of blood amino acids by the mammary gland but the quantity is entirely too small to account for the milk proteins Graham, Houchin, and Turner (1937) and Shaw and Petersen (1938a) did not simplify the problem by discovering that nearly sufficient urea is eliminated by the mammary gland to account for the nitrogen contained in the amino acids taken up Of course the source of the urea is not known Since Shaw and Petersen (1938b) described arginase in active mammary gland tissue one would expect some of the urea to be formed by the action of this enzyme on arginine

That blood globulin passes through the mammary cells in small amounts is unquestionable because of the identity of blood and milk globulin That large quantities are taken up is claimed by Graham, Peterson, Houchin and Turner (1938) and Reinecke, Peterson, Houchin and Turner (1939) Jackson and Gortner (1938) also suggest that blood globulin is a significant precursor of milk

proteins They found globulin to predominate in lactating while albumin predominated in nonlactating bovine mammary glands Reinecke, Williamson and Turner (1941) reported that appreciable glycoprotein material is taken up The evidence points strongly to blood globulin being a prominent precursor of milk proteins How it is converted into casein or lactalbumin or what part the amino acids play in the synthesis is not known It is significant that according to Shaw and Petersen (1938b) uric acid, creatine and creatinine are neither taken up nor eliminated

THE EJECTION OR "LET DOWN" OF MILK The phenomenon of "let down" of milk normally occurring as the result of the nursing or milking act has been subject to much speculation By many it was believed to be the result of a rapid secretion of milk brought about by the nursing stimulus (See section, "Time of Milk Secretion ") Others interpreted the phenomenon as being due to the forcing out of the milk from the alveoli and finer ducts Zwart (1916) was one of the first to draw attention to the distinction between milk secretion and the let down of milk but offered no explanation as to the mechanism involved in the latter

Failure properly to understand the phenomenon has led to a great deal of confusion As the nervous system is involved many have sought "secretion" nerves which Ribbert (1898) proved was not necessary for milk secretion as a transplanted rabbit gland secreted milk Yet many have sectioned the nerves to mammary glands in small animals and have noted adverse effects upon lactation (Ingelbrecht, 1935, Selye, 1934, Selye and Collip, 1936, Bacq, 1932, Selye, Collip and Thomson, 1932, Cannon and Bright, 1931, and Simeone and Ross 1938) Ely and Petersen (1941), however, noted no difference in the milk yield of the two halves of the cow's udder in one of which the nerve trunk had been cut and Labate sectioned all known nervous pathways to the ovary and uterus without any effect upon lactation Cannon and Bright noted the adverse effect to come in the next lactation following the operation Ingelbrecht resected the spinal cord in rats so that the front breasts remained innervated and made the significant observation that the young put on the posterior glands died from starvation when shielded from the sight of the mother When young were placed on the front glands simultaneously with those on the rear, both groups obtained milk Ingelbrecht concluded that most of the milk was secreted as a result of the nervous stimulation in nursing

That the nervous system is involved in lactation is not to be questioned but recent work has proven that its function is chiefly if not entirely concerned with the expression of the milk from the alveoli and finer ducts and not in its secretion Among the many theories advanced to account for the phenomenon is that of Zeitzmann (1922) who postulated a contraction mechanism in the gland as being responsible for retaining the milk Hammond (1936) postulated that the nursing stimulus reflexly caused engorgement of the gland with blood to squeeze the milk out of the alveoli The turgid condition of the gland at the beginning of nursing, held in support of this theory, is not due to engorgement with blood but rather to the distention of the lower portions of the gland with milk forced

out of the alveoli Ely and Petersen advanced the hypothesis that the "let down" of milk in cows was due to a reflex complex involving the sensory nerves in the skin of the teats and gland which when stimulated caused the secretion of the oxytocic principle of the posterior hypophysis, and this hormone in turn caused contraction of the musculature in the mammary gland described by Swanson and Turner (1941) Many have shown the posterior hypophyseal hormones to have milk expressing properties, beginning with Ott and Scott (1910) and later Turner and Slaughter (1930) and many others but Ely and Petersen (1939, 1941) were the first to advance the idea that the "let down of milk was caused by the oxytocic principle They noted that about 45 seconds intervened between the application of the stimulus and the let down of milk which agrees with the time required for the principle to be carried by the blood to the mammary gland in the cow Petersen and Ludwick (1942) demonstrated that the principle causing "let down' of milk was humoral in nature by showing that blood taken from cows stimulated to let down their milk caused "let down" of milk when perfused through excised glands while blood taken from cows not so stimulated failed In experiments using the perfusion technique, Petersen (1942c) added further evidence that the oxytocic principle is the causative agent While the pitressor principle caused "let down", also shown by Turner and Cooper (1941), it required larger quantities and had marked vasoconstrictor effects not noted in the blood from cows stimulated to let down their milk The observations of Miller and Petersen (1941) that incomplete evacuation of the gland resulted if milking was delayed after the stimulus for "let down" had been applied, also supports the claim that oxytocin is the active factor for it is well known that this factor is rapidly destroyed in the blood

The importance of considering this phase of lactation is obvious for if the milk is not removed from the gland the effects will be the same as when there is a failure of milk secretion That some of the so-called failures of lactation in experimental animals may be due to failure in the "let down" of milk is indicated by the report of Gomez (1939, 1940) who obtained milk secretion in post gravid hypophysectomized rats by the administration of extract of the posterior pituitary The reported successful lactation in rats by Smith (1932) and in dogs by Houssay (1935a) when the posterior hypophysis was removed is difficult to explain

As to be expected, Ely and Petersen (1941) found states of excitement may completely inhibit response to the milking stimulus It is not improbable that lactation failures, particularly in neurotic women may be due to a lack of response to the stimulus of nursing Knodt and Petersen (1944) have studied three cases of precipitous drops in the lactation in cows which were due to incomplete evacuation of the gland at milking as when the oxytocic hormone was injected, lactation levels were returned and maintained They also were able to level off the lactation curves of cows in the declining phase of complete evacuation of the gland at each milking by the use of oxytocin This suggests that the decline in milk yields and even the ultimate involution of the mammary gland may be due to a diminishing response to the milking stimulus and less complete evacuation of the gland That the mammary gland of the goat involutes in

about 30 days following suspension of milking was reported by Turner and Reinecke (1936) They also observed that involution was retarded in the side where milking was suspended when the other side was milked One explanation for this retardation may well be that with each milking there was a partial evacuation of the milk in the alveoli thus permitting limited secretory activity Additional support to the proposition that incomplete response to the milking stimulus is the cause of declines in the lactation curve is found in the report of Miller and Petersen (1941) that milk yields declined rapidly when evacuation of the gland was incomplete as a result of delay in milking following the stimulation for the "let down"

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# MAINTENANCE OF NITROGEN BALANCE BY THE INTRAVENOUS ADMINISTRATION OF PLASMA PROTEINS AND PROTEIN HYDROLYSATES

ROBERT ELMAN

*Washington University, St Louis, Missouri*

Studies of nitrogen balance have been used extensively in determining the effectiveness with which various forms of protein nourishment meet the protein needs of the body. Without going into a critical analysis of the accuracy of such a yardstick, it may be assumed that, apart from growth, the value of any protein administered for nutritional purposes must depend to a large extent upon whether it is able to maintain nitrogen balance. As a corollary to this metabolic point of view it may be further assumed that maintenance of nitrogen balance depends upon the presence of all of the essential amino-acids. The studies reviewed herein deal almost entirely with the administration of protein nourishment in the form of plasma protein or hydrolyzed protein through the intravenous route. The importance of this method has become apparent only in the past few years and will probably increase as the necessity of administering protein nourishment parenterally becomes more widely recognized. In order to evaluate correctly their influence on the level of nitrogen output, only studies will be included in which the injected material formed the *sole source of nitrogen*. As will be noted, this criterion has been maintained as far as protein hydrolysates are concerned, mostly for historical interest a few of the early studies on blood and plasma transfusions in humans and in animals were included in which protein food was also given by mouth. No attempt will be made to review a considerable literature on nitrogen balance following the parenteral, usually subcutaneous, injection of various serums and other proteins usually for purposes of immune or non-specific protein therapy, much of this data is old and has been reviewed elsewhere (29). Of historical interest (8) are a few early reports describing artificial nutrition, by which was meant the subcutaneous injection of milk and other substances, the term intravenous nutrition was apparently used even earlier (1899) in a report (27) which also contains much of this earlier literature.

In the following discussion various observations will be divided arbitrarily into those in which plasma and those in which hydrolyzed protein was injected, each group is further subdivided into observations made upon animals and upon humans.

PLASMA PROTEIN. Studies on the maintenance of nitrogen balance following the introduction of plasma protein intravenously include the use of whole blood as well as plasma transfusions. Fractions of the plasma proteins have been injected in the human but thus far no data on their metabolic behavior are available. In the case of whole blood the hemoglobin of the red cells represents over twice as much protein as that in the plasma part of the blood, yet it probably does not enter into the behavior of protein metabolism at least within the period of days usually involved in nitrogen balance studies inasmuch as hemoglobin is not catabolized as long as the red blood cell remains intact, and with compatible

blood, destruction of red cells is a slow process A few observations are included in which a solution of hemoglobin has been injected However, it is obvious that the injections of plasma alone are of greatest interest from the point of view of protein nutrition and most of the reports do deal therewith

*Animal experiments* Largely of historical interest are two studies reported in 1875 In the first (19) two fasted dogs were each given a large transfusion of defibrinated dog blood and the urine collected and studied for several days before and after, but only the urea output was measured A 10 to 20 per cent increase in urea was observed continuing for several days However, a control injection of 300 cc of glucose intravenously showed a similar increase, suggesting an untoward reaction, possibly from infected material These dogs were then given horse serum intravenously, but toxic symptoms occurred in two and there was an even larger increase in the urea output Egg white was given to one dog and it was followed by albuminuria In the second report (43) two dogs were given defibrinated blood, one by mouth, the other intravenously The urinary nitrogen was increased in each experiment, but much greater in the former than in the latter

The earliest modern study on nitrogen balance as influenced by the intravenous injection of plasma in animals was not made until 1908 (20) Both swine and dog serum were used, with the former, single doses in 7 dogs produced severe reactions and a pronounced negative balance due to a tremendous increase in nitrogen output in the urine, accounting for most or all of that administered Dog serum was then given in 3 single doses to 3 dogs and the nitrogen output followed for 5 days In 2 of the dogs which were fasted the nitrogen output did not change during the day of injection, even though the amount of nitrogen injected was only slightly greater than the total daily nitrogen output during control periods so that positive balance was achieved Moreover, there was no increase in output during the post-injection period In the third dog which was fed and already in positive balance, there was likewise no change in the urinary nitrogen during or after the injection, thus increasing the positive balance on the day of injection by the amount injected More variable findings are reported in another series of experiments (21) on 4 fasting dogs given single direct whole blood transfusions amounting to 100 to 200 cc In the first experiment the nitrogen output increased gradually so that it doubled on the 25th day after transfusion and then increased again for a day following a second transfusion In the second dog the nitrogen output remained unchanged for 2 days In the third animal it remained unchanged for 6 days and then fell In the fourth animal it increased after 5 days and then fell below the pre-injection level In still another dog, which was fed, nitrogen output increased gradually by 40 per cent on the 11th day No explanation was offered for the considerable variations, although no mention was made in regard to possible reactions Much more consistent findings were reported in another study (2) on 4 dogs kept on a nitrogen free diet and given serum intravenously on 3 successive days, the amount of nitrogen equalling the nitrogen output in the 3 previous days In 2 experiments with dog serum there was no change in the nitrogen output during the 3

days, thereby producing a positive balance, moreover there was no increase in nitrogen output during the 2 days following the injection In the other 2 experiments with horse serum there was an increased nitrogen output of about 20 per cent during the 3 days of injection in one case, but only a slight increase in the other instance during 2 days, positive balance was not observed in either case

Two other papers should be classified with these early studies, although they were performed in 1936 and 1938 In the first (28) six dogs were put on a non-protein diet and given small amounts (60 to 80 cc ) of citrated dog blood per day for from 1 to 5 days, and studied for 2 more weeks There was no change in the nitrogen output during the injection or post-injection periods except in one animal weighing 9 kgm given a larger amount of blood (130 and 155 cc on 2 successive days) However, even here the increase was very slight and occurred on the second day, the amount of nitrogen in the plasma was, however, insufficient to expect positive nitrogen balance In the second paper (39) similar experiments were carried out on 3 dogs kept on a low nitrogen diet previous to the injection of whole citrated blood In only one of these experiments was there a significant increase in the nitrogen output during the days in which a transfusion was given Changes after the transfusions were finished were hard to estimate in view of the fact that the animal was put on a full diet at the conclusion of the transfusion period

The probable influence of incompatible blood on nitrogen balance formed the subject of a study in 1932 (24) in which whole dog blood was given to dogs kept on a protein-free diet Experiments were divided into 2 groups depending upon whether the blood was absolutely compatible or whether it showed evidence of incompatibility The experiments were carefully carried out for several days before and after a single transfusion of 100 cc of whole blood With this amount it was impossible to expect positive nitrogen balance, calculating as nitrogen intake only the plasma protein However, the difference in the nitrogen output in the 2 groups was significant Seven experiments with blood which did not match resulted in a 10 to 20 per cent increase of the urinary nitrogen output developing within a few days, but which returned to normal in 6 to 8 days By contrast there was not only no change in the nitrogen output in the urine, but indeed a slight fall in 5 experiments, with transfusions of blood which matched completely In each group both citrated as well as direct transfusions were employed with similar results

In the past decade there has been an increasing number of studies on nitrogen balance in dogs given plasma or serum intravenously In 1934 experiments were described (23) in which heparinized dog plasma was injected into 4 dogs on a nitrogen-free diet In the first dog negative balance occurred during the first of two 7 day injection periods, even though the nitrogen injected exceeded the output during the preliminary 5 days, however, in the second 7 day period a positive balance of nearly one gram was observed because the nitrogen output fell in spite of an increased intake, in the post-injection period of 5 days there was only a slight increase in the nitrogen output In the other 3 dogs consistent positive

balances were achieved in 2 successive 7 day periods with no increase in the post-injection period of 5 days By contrast a similar experiment in which the plasma was given by mouth rather than vein was followed by negative balance due to an increased nitrogen output, though the excretion in the final control period dropped to the same level as that of the preliminary control period In a paper the following year (37) further similar experiments were reported from the same laboratory In the first of 2 experiments nitrogen balance was barely missed during two 7 day periods and one 4 day period, due undoubtedly to the small dose of plasma given, in none of them did the nitrogen output reach the preliminary level In the second experiment nitrogen balance was achieved during one 8 day injection period, in the 5 day period following the nitrogen output fell By contrast, however, one experiment is described in which intravenous horse plasma for one 7 day and one 3 day period led to a progressive increase in the nitrogen output lasting into the post-injection period, moreover the dog became sick and died and at autopsy showed a definite nephritis In another experiment dog plasma was injected intraperitoneally on each of 8 days without any increase in the nitrogen output, producing a positive balance of over one gram a day, however in the 5 days following injection there was an increase of over 25 per cent in the nitrogen output Horse serum given intraperitoneally in another experiment resulted in a slight increase in the urinary output of nitrogen during the 7 day injection period, a fall in the first and an increase in the second of two 4 day post-injection periods Nitrogen balance was not achieved, however, the dose of nitrogen was much less than the output during the preliminary period Hemoglobin was given intravenously in one experiment in amounts of less than half of the nitrogen excreted during the preliminary period Nevertheless, a 25 per cent increase in the nitrogen output occurred during two 7 day periods which fell in the 5 day post-injection period to a much lower level Hemoglobin given intraperitoneally in one experiment in larger amounts almost equal to the nitrogen output in the preliminary period resulted in a 50 to 75 per cent increase in the nitrogen output during 2 periods of 7 and 4 days respectively However, in the post injection period of 4 days the output fell almost to the preliminary level

The probable influence on nitrogen balance of untoward reactions following plasma transfusions is revealed in subsequent similar observations from the same laboratory (10) Three intact dogs were studied while on a non protein regime In the first animal positive nitrogen balance was maintained in 7 out of 9 two day periods during which the amount of plasma injected each day was but slightly larger than the level of output in the preliminary period In the entire 18 days the total positive balance was +3.2 grams Moreover, in the 3 two day periods following cessation of the transfusions there was a definite fall in the output of nitrogen below the preliminary level However, in the second dog during 4 two day periods plasma injections produced an increasing output of nitrogen in the urine resulting in an astonishing negative balance of over 30 grams, or almost 5 grams a day even though the total nitrogen injected was a little over half of the amount excreted during the preliminary period That the plasma produced intoxication was shown by rapid loss of weight during this period and the excre-

tion of tremendous amounts of creatin and uric acid in the urine. During 7 out of 10 subsequent 2 day periods, this negative balance changed to a positive balance presumably because the dietary intake was altered from plain sugar by mouth to a fuller but still non-protein diet. Nevertheless, the positive balance during this second period was not very great, 2.4 grams for the entire 20 days, although the intake was increased during this period. In the third dog plasma injections also produced evidence of intoxication as shown by frequent vomiting even though there was no increase in the output of creatin and uric acid. A negative balance during each of 7 two day periods totalled 12.4 grams for the 14 days, or nearly a gram per day. The amount of plasma injected each day was somewhat larger than the nitrogen excreted during the preliminary period. The intake of food in this experiment consisted entirely of sugar by mouth, however, during 3 two day periods after discontinuance of the injections, the administration of plasma and sugar by mouth was followed by a fall in the nitrogen output to the preliminary control period.

In another paper (32) from the same laboratory two dogs on a low protein diet were each given plasma for 2 days without provoking any increase in the nitrogen output in the urine even though the amount injected was greater than the preliminary nitrogen output, thus achieving a pronounced positive balance. In still another report (31) plasma was injected during two 7 day periods in dogs being depleted by plasmapheresis on a protein-free diet. A positive balance of 6.3 and 3.3 grams was observed during these periods even though the amount of nitrogen injected was not much greater than the previous output. However, the urinary nitrogen increased 3 and 4 times during the subsequent periods due partly, if not entirely, to a corresponding increase in the oral intake of protein. In still another report (38) dog plasma was given to an Eck fistula dog on a low protein diet, kept anemic by repeated bleedings. During two 7 day periods 20 grams of nitrogen were retained, or almost 1.5 grams a day on an intake of less than 3 grams a day as plasma. Even if the output of nitrogen in the hemoglobin and plasma of the blood is subtracted, a good positive balance was achieved.

Other workers have described somewhat similar experiments in which much larger amounts of plasma were injected (15) in dogs rendered hypoproteinemic by three weeks of a non-protein diet. During the fourth week daily injections of citrated dog plasma were given totalling about 5 grams of nitrogen per day or about 3 or more times the previous level of nitrogen output in the urine. During this week of injection the nitrogen output remained unchanged over the preliminary control period so that a remarkably positive nitrogen balance of nearly 3 grams a day was achieved. However, during the subsequent 2 weeks during which there were no injections the nitrogen output increased remarkably, wiping away much of the nitrogen retained during the injection period. By contrast, similar experiments in which hydrolyzed protein was injected as described later (17) were not followed by such an increased urinary output of nitrogen.

*Studies in the human.* The earliest study of nitrogen balance following intravenous administration of blood in humans was apparently reported in 1921 (7). Four patients with pernicious anemia were observed during many 3 day

periods. In one period daily transfusions of 350 to 500 cc. of defibrinated whole blood were given, alternating with periods in which no blood was injected. The patients were on controlled full diets and in positive nitrogen balance. Findings were variable but there was sufficient increase in the nitrogen output during the transfusion periods to produce slight negative balance if one calculated as nitrogen intake only the plasma of the transfusion and not the hemoglobin in the red cells. On analyzing the data, however, it seemed significant that the largest nitrogen output in the urine occurred during days in which the transfusion had produced a febrile reaction, whereas in one patient showing practically no febrile reaction, there was no change in nitrogen output during the transfusion periods, thus increasing the degree of positive balance. In contrast to these findings is another study a few years later (45) in which 6 patients with pernicious anemia were given a single transfusion of 500 to 750 cc. of blood and the urinary nitrogen observed for one day before and many days afterwards. The food nitrogen intake was kept constant throughout by a regulated standard diet; however, in the one day preliminary measurement only 2 of the 6 patients were in positive balance before the transfusion, the other 4 being in slightly negative balance. The transfusions in all cases produced a negative balance even if one took into account the nitrogen of the injected plasma due to an increased output of nitrogen which reached its highest point on the second or third day, but returned to normal on the fifth day. The magnitude of this increase varied between 2.4 grams to 4.6 grams of nitrogen per day over the control output of slightly over 6 grams. There was also a coincident increase in the uric acid excretion. No mention is made as to the manner in which the blood was given, whether it was citrated, or as to the presence of reactions. That no hemolytic reactions occurred is indicated by the fact that a well sustained increase in red cell count and hemoglobin was recorded in each case. The reported values for plasma proteins were normal or slightly high previous to the transfusions and showed no change afterwards. At about the same time another study appeared in which 6 infants and children were given transfusions of whole blood, washed red cells or plasma, all citrated (36). It is difficult to evaluate the data in this study particularly if an attempt is made to discount the effects of untoward reactions which apparently did occur; for example, following one whole blood transfusion hemoglobinuria was observed. Moreover, no data on nitrogen balance can be obtained, inasmuch as only the urea of the urine was determined. Nevertheless, it is of some interest to note that the output of urea in the urine following the injection of plasma was definitely increased in at least 2 cases. In one of these, a 17 months old infant, there was a rise from 0.80 gram per day on 4 successive days after the injection of 55 cc. of plasma containing 0.590 gram of nitrogen, returning to normal after an excess of 0.642 gram of urea had been excreted or more than the total amount of nitrogen that had been injected. However, in another instance in a one year old infant there was no change in the urea output after the plasma transfusion. In 2 instances red cell suspensions were injected without any change, whereas in 3 instances the injection of whole blood was followed by a slight increase. Following the injection of 60 cc. of saline as a control there was

no change in the output of urea for 3 days, though the authors stated that the total nitrogen did increase

The next report appeared quite recently (25) and describes 2 cases which were studied before, during and after daily injections of bovine and human plasma intravenously In the case of the bovine plasma the daily injection of 6 15 grams of nitrogen for 5 days resulted in an output of 6 91 grams or a negative nitrogen balance of 0 76 gram The caloric intake as glucose during the entire experiment was 1500 per day and no food was taken by mouth Although there was a slight negative balance the output of nitrogen did not increase and indeed was below the 7 88 gram level of the preliminary 5 days, and was only slightly above the post-injection 6 45 gram level of 5 days Thus a positive balance may have been achieved with slightly larger injections On the other hand, the patient in whom human plasma was injected went into a pronounced daily positive balance of +1 23 grams during the 5 days in which plasma containing 5 6 grams of nitrogen was injected The output during the preliminary period was 6 62 grams and the post-injection period of 4 days was 3 39 grams In other words, the plasma transfusions were accompanied and followed by an actual fall in the urinary excretion of nitrogen

INJECTIONS OF HYDROLYZED PROTEIN In the following observations the proteins used were digested by different methods and include a few studies with mixtures of pure amino-acids A recent review (33) on intravenous alimentation with amino-acids contains a discussion of the different types of protein hydrolysates Observations on the metabolic effects of injections of single amino-acids will not be reviewed, discussion will be confined to studies made with hydrolyzed protein or mixtures of amino-acids given intravenously for nutritional purposes and will be divided into animal and human observations

*Animal experiments* As early as 1889 an undetailed report (35) appeared in which a digest of casein and other proteins, prepared by an apparently mild hydrolysis with alkali was injected intravenously without producing albuminuria or toxic symptoms, which had been noted following the injection of whole casein solutions However, nitrogen balance was apparently not achieved inasmuch as the administration was followed by an increased output of non-protein nitrogen in the urine In the paper on nitrogen balance following plasma injections, already discussed (29), are described 2 experiments in which an alkali hydrolysate of milk protein was injected into dogs with some evidence that much of the injected nitrogen was retained though not enough was given to expect positive balance This was in 1908 Four years later (6) an enzymatic hydrolysis of meat was used which, however, evoked violent reactions including diarrhea and albuminuria when injected intravenously in dogs and a pronounced negative nitrogen balance The following year a successful experiment was reported (22) in a 15 5 kgm goat which was maintained in positive nitrogen balance for 16 days, all fluid and food were given by a continuous intravenous drip containing glucose, salts and an enzymatic hydrolysate of meat in which 10 to 15 per cent of the nitrogen was still present as peptides The amount of nitrogen injected was 5 6 to 8 4 grams a day, or somewhat above the preliminary 2 day excretion

of 5 8 and 4 5 grams during which glucose alone was given, the positive balance varied between 0 65 to 2 1 grams a day Of special interest was the fact that the peripheral veins were used for the venoclysis for 10 days, when it became necessary to operate and introduce the cannula into the splenic vein, which was used for the last 6 days, during the latter period there was no increase in the degree of positive balance thus indicating that nitrogen retention was just *as good whether hydrolyzed protein entered the portal or systemic blood* The goat died on the 18th day and at autopsy showed no infection, but a massive thrombosis was found extending from the jugular vein to the vena cava The same authors achieved positive nitrogen balance of 1 6 to 4 grams in 2 dogs for 4 days using the same hydrolysate Both dogs died on the fifth day The same year (44) another report appeared in which hydrolyzed meat and casein were injected intravenously in dogs but the experiments were of short duration and not designed to study nitrogen balance Nevertheless, they represent a milestone in our knowledge of amino-acid metabolism

No further work was done for over two decades In 1938 (11) an acid hydolysate of casein was injected intravenously, only when tryptophane and cystine were added thereto did immediate positive balance occur Three day periods were studied in dogs maintained on a nitrogen free diet and the amount of nitrogen injected was but slightly more than the output during the preliminary period of 3 days before injection Conversely, when the 2 amino-acids were omitted, immediately, i.e , by the very next day, the animal went into negative nitrogen balance Further experiments (12) with this supplemented acid digest of casein showed that nitrogen balance depends upon whether the added tryptophane and cystine were introduced at the same time as the acid hydrolysate or were injected 6 hours later Curiously enough even such a short delay in the injection of the added amino-acids resulted in a negative nitrogen balance Thus it would seem that the maintenance of nitrogen balance with intravenous administration is dependent upon, or at least greatly influenced by the simultaneous presence of all of the essential amino-acids in the injected fluid

An interesting and perhaps important aspect of these two last observations is the rapid time relation of injected nitrogen on the nitrogen output, unlike the delay usually observed when protein is given orally Thus the step-like increase or decrease in nitrogen output following the addition or withdrawal of protein repeatedly observed by many workers on nitrogen balance with oral administration is apparently not present with intravenous administration of hydrolyzed protein The fact that much of the theory of protein stores in the body rests upon this behavior of the nitrogen output following orally administered protein gives theoretical significance to these findings Inasmuch as this step-like phenomenon was not observed and has not been observed by others in subsequent intravenous studies would seem to indicate that the delayed effect of protein given by mouth on nitrogen output has something to do with a lag in digestion and absorption of protein in the gastrointestinal tract and not with the presence of any body store of protein or, as it is often called, "deposit nitrogen " Moreover, the idea that nitrogen balance may be affected by the presentation of all

essential amino-acids to the tissues *at the same time* may also explain some of the delay in the effect of oral administration of protein as compared with intravenous injections.

Several years later from the same laboratory studies (17) were reported following the injection of a protein digest made by the hydrolysis of casein by pork pancreas, a method of preparation which did not destroy tryptophane and which yielded a product containing also the amino-acids present in the pancreatic protein used. While this digest required no amino-acid supplement, unlike the acid hydrolysate, it was incompletely digested in that about 30 per cent of the protein was still in the form of small peptides. In 5 dogs this hydrolysate was given intravenously each day as a 5 per cent solution plus 5 per cent glucose during the fourth week of the study, the first 3 weeks and the fifth week being control periods. Practically no protein was given by mouth during the entire 5 week period, the amount of nitrogen injected during the week of injection averaged 23 grams of nitrogen, or about 3 times the average nitrogen output during the week before and the week after the injection. (There was no increase in nitrogen output during the week following the injection.) During the fourth or injection week the output was 17.8 grams, giving a positive nitrogen balance of 5.2 grams. Of the nitrogen output during this week of injection 7.3 grams, or the control output, would still have been excreted and thus the rest, or 10.5 grams (17.8−7.3) therefore originated from the injected digest, indicating that 54.6 per cent $\left(\frac{23 - 10.5}{23}\right)$ of the nitrogen supplied was actually retained and utilized by the body. Four similar experiments in which the same amount of hydrolysate was given by mouth showed a 56.6 per cent utilization, thus indicating that maintenance of nitrogen balance was of a similar degree whether the digest was given by mouth or intravenously.

In a study designed to gain information regarding the effect of large injections of protein digests (14) 3½ grams of nitrogen per kilogram of body weight were injected during 24 and 48 hour periods in 6 dogs depleted by a three week non-protein diet. Although positive nitrogen balance was obtained during the period of injection, large amounts of nitrogen appeared in the subsequent days in four experiments in which this amount of nitrogen was injected during 24 hours. However, in the 2 animals in which the injection of the digest combined with glucose was spread over a 48 hour period, positive nitrogen balance was achieved without subsequent loss during the 7 day period following the termination of the injections. This indicated that the injection of 1.7 grams of nitrogen per kgm. per day for 2 days resulted in the same degree of nitrogen retention as the same amount of nitrogen spread over a period of 7 days, and suggested an approximate ceiling of nitrogen utilization, which corresponds to a daily protein intake of about 10 grams per kgm. per day.

Other digests have been investigated. A papain digest of casein was used (32) in 5 dogs and nitrogen balance studied following the injection of a 5 per cent solution thereof with 5 per cent glucose in amounts slightly greater than the basic excretion of nitrogen on a non-protein regime. In 3 dogs depleted by

plasmapheresis the study was made for consecutive 7 day periods In the first dog a positive nitrogen balance was obtained for 5 out of 7 such periods and for all of the succeeding 6 periods, i.e , for 13 continuous weeks with 2 exceptions In the second dog 4 similar periods resulted in positive balance of +0 5 and +2.3 grams during 2, and negative balance of −1 7 and −1 5 grams during the other 2, although in 2 subsequent periods in which the digest was injected subcutaneously, a positive balance of +1 7 and +4.3 grams was achieved In the third dog the digest plus tryptophane and cystine resulted during one period in a slightly negative balance of −0.8 gram In the remaining 2 animals nitrogen balance studies were carried out on dogs not subjected to plasmapheresis but merely maintained on a low protein diet During 4 two day periods negative balance was observed as high as −4 6 grams In a subsequent 2 day period the addition of tryptophane and in another 2 day period the addition of tryptophane and cystine to the digest was still followed by a negative nitrogen balance, although during the following 4 days the same mixture by mouth resulted in a positive balance of as much as +2 1 grams The same observers also studied another digest of casein which was described as a mixture of acid and alkali hydrolysates When injected into one dog depleted with plasmapheresis during two 7 day periods a markedly negative nitrogen balance of −10 9 grams and −5 6 grams occurred Subsequently, during a 7 day period this digest, supplemented by cystine and tryptophane, was injected, resulting in a +3 0 grams positive balance In 2 dogs not subjected to plasmapheresis this hydrolysate supplemented with cystine and tryptophane yielded one 2 day period of positive nitrogen balance of +0 49 gram, the rest being negative, up to −1 09 grams In another report (38) from the same laboratory papain digests of casein and beef serum were injected intravenously in dogs maintained on a protein-free diet, but kept anemic by repeated small bleedings There were 5 observations, in the first two of which beef serum digest was used In one dog during three 7 day periods a positive nitrogen balance was observed of 20 grams which would be lessened but not eliminated even if both the hemoglobin and plasma removed during this period were subtracted In the second experiment involving two 7 day periods the positive balance was nearly one gram a day, which would be cut in half if the amount of hemoglobin and plasma removed in bleeding were subtracted In the next 3 experiments a casein digest was used In the first of these during two 7 day periods there was a positive balance of +9 grams, which however, would be turned into a negative balance only if both the hemoglobin and plasma removed during this period were taken into account This was true also of the next experiment in which during two 7 days periods a positive balance of +16 1 was achieved In the final experiment a positive balance of +12 grams was achieved during one 7 day period, which would be lessened if both the hemoglobin and plasma removed during the period were subtracted

Nitrogen balance has been studied following the injection of a gelatin solution (4) which in reality is a protein hydrolysate, inasmuch as it is usually prepared by boiling animal tissues a process which dissolves and digests the water soluble proteins into particles of varying size however it is probable that little of it is

present as amino-acids In 4 dogs, 6 periods varying between 7 and 15 days showed that in all but one a slight positive balance was achieved However, the amount of nitrogen injected was 4 to 13 times the amount of nitrogen excreted during the control periods Moreover, partial data from subsequent experiments indicated that in the period following the injection of gelatin large amounts of nitrogen appeared in the urine, suggesting that the positive nitrogen balance observed during the actual period of injection represented a retention which was subsequently lost Gelatin, of course, does not contain all of the essential amino-acids and should not, therefore, be able to maintain nitrogen balance

Various mixtures of pure crystalline amino-acids have also been injected for purposes of protein nutrition In the first experiments along this line (30) the mixture used contained only essential amino-acids plus glycine and arginine Seven of the amino-acids used were racemic The amount of nitrogen injected was the same as in previous periods during which similar mixtures by mouth resulted in positive balance The dogs were being simultaneously depleted by plasmapheresis but were on a non-protein diet In the first of two 7 day periods a negative balance of −5 4 grams was observed but is probably explained by carry-over of the effect of a deficiency in threonine in the mixture injected during the preceding period, in the second 7 day period, moreover, a positive balance of +0 5 gram was achieved In a subsequent paper by the same authors (38) a similar mixture of amino-acids was injected intravenously for 7 days in a dog kept on a non-protein diet, but bled frequently to produce anemia A positive balance of +11 6 grams was observed during the period not calculating the amount of nitrogen removed as hemoglobin and plasma which, however, totalled but 3 7 grams In another laboratory (16) a mixture containing only the 9 essential amino-acids were injected in dogs taking only sugar by mouth, which showed that nitrogen balance could be achieved thereby during 3 day periods following the injection of an amount of nitrogen which was only slightly more than the amount excreted during the control periods Of special interest was the observation that the omission of histidine from such a complete mixture was still followed by positive nitrogen balance for a period of 3 days, but that a negative balance followed during 2 subsequent 3 day periods, suggesting that histidine is relatively stable and, unlike tryptophane, requires a longer time before its absence is manifested in nitrogen balance experiments This lag in the production of negative balance was also observed in the experiments on the intravenous injection of gelatin just mentioned, and in one of the reports on plasma transfusions As will be discussed later, these observations add further support to the idea that important time factors must be considered in all studies on nitrogen balance

*Observations in the human* Though first used but 6 years ago, many observations have already been made on the maintenance of nitrogen balance in the human following the intravenous injection of various hydrolysates of protein This discussion will be confined to studies in which the injected digest formed the *sole source of nitrogen* No observations will be included in which these injections were combined with the oral administration of protein food because of obvious difficulty in evaluating the relative influence of the two sources of nitrogen on nitrogen balance

It was only in 1938 that hydrolyzed protein was injected intravenously into the human for the first time (18) Positive nitrogen balance was achieved with an acid hydrolysate of casein supplemented by tryptophane and cystine Eight cases were described, although detailed data were mentioned in only 2 instances One, a 67 year old woman, unable to take anything by mouth because of an inoperable carcinoma of the stomach, was maintained with saline and glucose with a level of nitrogen output in the urine of 4 grams a day, on 3 successive days positive balance followed the addition of 4 5 grams of nitrogen as the protein digest In another case, a 69 year old woman with severe septicemia, in coma, exhibiting nutritional edema, 5 16 grams of nitrogen as the casein digest was added to the intravenous drip of saline and glucose for 3 days The urinary nitrogen previously 4 grams a day, increased to 4 5 grams a day, thus producing a positive balance of +0 66 gram a day for the 3 days In a subsequent report (13) further clinical experiences were described in which an enzymic hydrolysate of casein was used, requiring no addition of supplementary amino-acids This preparation was made by the digestion of casein with pork pancreas and therefore contained the amino-acids present in the pancreas protein as well as in the casein This digest, moreover, unlike the acid hydrolysate which contained no peptides, was incompletely broken down so that about 30 per cent of the nitrogen was present in the form of small peptides The term Amigen has been applied to this last preparation by the manufacturers and for simplicity will be used in the following discussion This report described the use of Amigen in 35 patients, of which 8 cases were described in detail During a preliminary period a solution of 5 per cent glucose in saline was injected and compared with subsequent periods in which the hydrolyzed protein was merely added to the same amount of glucose In many of the cases there was a second control period after the injections Positive nitrogen balance was achieved in 4 of the 8 cases partially so in 2 and incompletely in 2 The daily amount of nitrogen given did not exceed 9 6 grams which was somewhat more than the output during the preliminary period in the first 4 cases mentioned, but considerably less than the output in the second 4 cases which were more seriously ill patients and probably because of this, showed an unusually high nitrogen loss Failure to achieve consistent nitrogen balance in the last 4 cases may be attributed to the fact that an insufficient amount of nitrogen was injected There is little evidence in these experiments that the introduction of the digested protein of itself led to a significant increase in the nitrogen output For example, in the first case, a relatively normal individual, the nitrogen output in 3 preliminary control days averaged about 5 5 grams, for 12 subsequent days, during which 7.2 grams of nitrogen was added to the glucose the positive balance varied between 1 and 3 grams a day In the second case the output during the control period was somewhat higher, averaging about 6 5 grams a day, yet positive balance was also achieved although it did not exceed one gram a day

Other observers used the same hydrolysate in 30 postoperative patients (20) with similar results The solution contained 3 per cent Amigen and 7 per cent glucose and was injected for an average of 7 days during which no food was ingested The amount of nitrogen injected was 7.2 grams per day Details of

nitrogen balance were described in 2 patients In one, following a simple appendectomy without complications, positive nitrogen balance of +1 4 to +3 1 grams per day or a total of +14 6 grams during 6 days was achieved In the second patient, who had peritonitis, the same amount of Amigen did not result in positive balance, indeed there was a negative balance of −2 1 to −12 9 grams a day These findings were stated to be characteristic of the other cases, i e, positive nitrogen balance could be obtained with this dose of Amigen nitrogen only when the patient was excreting less than this amount, but not in the sicker patients with higher losses in the urine, which in one case amounted to 25 grams of nitrogen in one day Another observer (9), using the same digest, was able to produce positive nitrogen balance in a 57 year old male who was studied for 18 days during which nothing was taken by mouth, data during 13 days in which urine collections were complete showed that the injection of Amigen in amounts varying between 6 and 12 grams per day with added glucose and a fat emulsion resulted in a positive nitrogen balance of between +0 5 and +5 2 grams a day in all but 2 days During these 2 days the negative balance was −1 8 and −1 6 grams of nitrogen In general the highest positive balance was observed when the largest dose, i e, when 12 grams of nitrogen, was injected In a subsequent report (3) this same observer studied an adult female for 6 days during which the patient received nothing by mouth, the injections containing 18 grams of nitrogen as Amigen plus 300 grams of glucose A positive balance of 23 3 grams of nitrogen was observed during this 6 day period or nearly 4 grams a day In another more detailed study (5) from the same clinic data are described on 9 patients, all of them operated upon and showing huge losses of nitrogen in the urine, undoubtedly as a result of their disease A positive nitrogen balance in general was not consistently achieved with the amounts injected, probably because of the large loss of nitrogen in the urine, although in one patient for a period of 10 days during which 18 grams of nitrogen was injected each day, a positive nitrogen balance of +48 84 grams was observed, or nearly +5 grams a day

Nitrogen balance studies (26) following the injection of Amigen solution as the sole source of nitrogen were reported from still another clinic, the details of 2 cases being described In the first case a patient with squamous carcinoma of the lower end of the esophagus was first studied for 6 days before operation Following a control period of 3 days in which no nitrogen was injected, positive nitrogen balance was achieved in the next 2 days, during each of which 12 grams of nitrogen were administered On the sixth day 6 grams of nitrogen were administered with a negative nitrogen balance At operation the lower end of the esophagus and cardia of the stomach were resected, followed by a primary anastomosis On the second day after operation, during which the patient received only saline and glucose, 15 6 grams of nitrogen appeared in the urine On the next day 9 grams of nitrogen as Amigen were injected with a positive balance of +1 7 grams On 4 subsequent days the nitrogen balance was negative (−0 1 to −5 1 grams a day) on an intake of Amigen of but 6 grams On 2 subsequent days during which no nitrogen was injected the output was 10 13 grams per day

In the second, a non-operative case, between 6 and 12 grams of Amigen nitrogen were administered each day as the sole source of nitrogen for 8 days. During this period there was a total positive nitrogen balance of +6.7 grams, or an average of nearly a gram a day.

In a series of reports in infants, Amigen was injected intravenously for varying periods of time as the sole source of nitrogen. In the first study (42) six 2 day periods were studied on 4 infants and one older child, a positive nitrogen balance being obtained in all but one case, the degree of which was calculated as varying between 3 and 46 per cent of the amount injected. These figures were then compared with similar periods in which the same amounts of nitrogen were ingested as a milk formula and the degree of retention was practically the same. In a second study (41) the observations are of interest even though not designed to give information as to nitrogen balance. The nitrogen excretion in the urine was compared in infants during five separate 24 hour periods in which Amigen was injected with two 24 and one 12 hour period in which a mixture of crystalline amino-acids made up to contain the estimated composition of casein. Although nearly one-fourth of the amino-acids of the crystalline amino-acid mixture was in the unnatural form, the excretion of nitrogen in the 2 groups of observations was entirely similar. In a third and more detailed study (40), 20 male infants were given Amigen intravenously and compared with control periods in which only glucose and saline were injected. Positive nitrogen balance was achieved when the nitrogen intake exceeded 0.31 gram per kgm. per day but was greatest when the intake was 0.92 gram per kgm. per day, the latter amount producing a positive balance of +0.50 gram per kgm. per day i.e., 54 per cent of the intake. These figures may be calculated in another way by using as a baseline the control output of nitrogen which is given as −0.15 gram per kgm. per day. In the first series of observations during which 0.50 gram per kgm. per day was given the output was 0.34 gram. Now 0.15 gram may be subtracted from 0.34 gram as representing the control output, which gives 0.19 gram as representing that part of the output originating from the injected digest, which when subtracted from 0.50 gives 0.31 as the nitrogen actually retained and this is 62 per cent of the amount injected. Similarly, at the higher level of injection of 0.92 gram during which the output was 0.42 gram, a similar figure of 70 per cent retention may be obtained. It will be also observed that twice as much nitrogen had to be injected as was excreted during the control period in order to achieve nitrogen balance.

An acid hydrolysate of casein has been used in other observations. Tryptophane was added to make the mixture complete, inasmuch as such hydrolysis was followed by destruction of this amino-acid. Earlier observations with a similar hydrolysate have already been mentioned (18). In one adult (1) such a hydrolysate was used as the sole source of nitrogen after operation for intestinal obstruction but nitrogen balance was not achieved. During a period of 8 days there was a daily negative balance of −3.58 grams with an intake of 5 grams which was greater than the output of 3.96 grams during the control period of 4 days during which glucose and saline alone were injected. Very large amounts

of creatin were excreted in the urine during the period in which the digest was injected  Successful achievement of positive balance, however, was achieved with the same hydrolysate as the sole source of nitrogen by another observer (34) in a patient operated on for pelvic abscess  There was a preliminary control period of 5 days in which there was no nitrogen intake  Following this was a 6 day period in which the nitrogen intake consisted of transfusions of blood as well as injection of the casein digest, and positive balance was observed  However, during a third 5 day period the digest formed the sole source of nitrogen, 12 to 18 grams a day were injected intravenously and daily positive balance of +1 1 to +4 9 grams was observed

COMMENT  Maintenance of nitrogen balance, as shown by the studies here summarized, can be achieved both with plasma transfusions and with the intravenous injection of mixtures of amino-acids either as pure crystals or as hydrolyzed protein  In the case of plasma, compatibility is a prerequisite for there is sufficient evidence to show that even plasma from the same species will lead to negative balance if there is an untoward reaction  Even in the absence of such reaction, there is some evidence that positive balance may require donor plasma which is of the same type as the recipient, although much further study is needed  The influence of the added anticoagulant may also prove of significance

In the case of amino-acid mixtures, the presence of all of the essential ones in the injected solution is a clear prerequisite for maintenance of nitrogen balance at least for periods of more than 3 days  With injections of hydrolyzed protein, failure to maintain balance with some preparations is undoubtedly also referable to a deficiency in one or more of the essential amino-acids  The influence of untoward reactions following the intravenous administration of hydrolyzed protein is probably the same as in the case of plasma, although no observations are available

In several of the studies there are indications that glucose should be added to the solutions of hydrolyzed protein in order to achieve nitrogen balance more effectively  While the importance of glucose seems obvious on theoretical grounds, there is insufficient data to show just how important this consideration may be  It is hoped that further studies will be made with varying proportions of glucose in order to show whether glucose is really required, and if so, what the optimum amount may be

Further consideration of the data reviewed herein invites 2 comparisons regarding maintenance of nitrogen balance with intravenous injections of nitrogenous nourishment  First are differences between this method and the oral one, second are differences between the intravenous injection of whole protein as plasma transfusions and the injection of the building stones of protein as hydrolyzed protein or mixtures of amino-acids  Inasmuch as protein nourishment normally enters the blood stream as amino-acids or small peptides, it is obvious that a plasma transfusion introduces entirely new metabolic problems as compared with the ingestion of the same plasma proteins by mouth  In other words, the introduction of whole protein molecules directly into the blood stream has no physiological counterpart in the normal mechanisms of nutrition  That the

injected protein leaves the blood stream rapidly and to a great extent seems clear On theoretical grounds, however, it is difficult to predict whether the injected plasma protein which leaves the blood stream is utilized as such by body tissue, whether it is broken down to smaller units, even to amino-acids by cellular proteases or indeed whether it simply undergoes an overhauling process by the interchange of free amino-acid groups without hydrolysis before being used elsewhere The evidence herein reported, though limited, does indicate that nitrogen balance is more readily achieved by the intravenous injection of plasma protein than by its oral ingestion thus indicating more efficient utilization in the former case On the other hand, longer observations are probably necessary in view of the fact that plasma protein breakdown is a slow process Observations with isotopic nitrogen indicate that the half life of plasma protein is about 14 days Nitrogen balance studies following plasma transfusions, therefore, should probably be continued for 2 or more weeks in order to determine whether the nitrogen injected is really retained permanently or whether there is merely a delay in its excretion A time factor is also important in the behavior of hydrolyzed protein injected intravenously, as already discussed It is suggested, moreover, that growth experiments be also carried out with plasma protein inasmuch as important evidence of the amino-acid composition of plasma protein can be obtained in this way which may be of value in correlating data on nitrogen balance

The above mentioned differences between the oral and intravenous administration of plasma do not apply to hydrolyzed protein inasmuch as all protein food is normally hydrolyzed before absorption Thus the injection of appropriately hydrolyzed protein or amino-acid mixtures introduces no new or unusual physiological mechanism except for the fact that the material enters the systemic rather than the portal circulation Although the liver is probably the most important organ in the metabolism of amino-acids, other tissues, of course, do play an important part While there is no theoretical reason why there should be any difference in utilization between amino-acids entering the portal vein and the systemic veins, the rather limited evidence, as reviewed herein, does with one or two exceptions, point to the similarity in the nitrogen balance produced by the two modes of administration An analogy may be drawn by citing the fact that the intravenous injection of glucose leads to glycogen storage in the liver as well as does oral administration

The inclusion of protein nutrition in parenteral injections now extensively used for patients unable to take anything by mouth is but a natural extension of this mode of therapy which for years has been confined to the administration of saline and glucose solutions plus certain vitamins It is probable that this method of treatment will be used more and more as the importance of preventing or correcting protein deficiencies in this way becomes more generally realized Acute starvation in patients unable to eat requires the parenteral administration of a more or less complete diet and this, of course, is impossible unless nitrogenous nourishment is included Much more investigation must be done before the metabolism of intravenously injected protein nourishment is fully understood,

particularly regarding the injection of the larger molecules and especially the whole protein molecules in a plasma transfusion

## SUMMARY

Review of the literature through 1943 reveals sufficient evidence of the fact that nitrogen balance may be achieved both in animals and man by the intravenous administration of compatible plasma as the sole source of nitrogen Nitrogen balance can similarly be maintained by the intravenous injection of solutions containing mixtures of all of the essential amino-acids or certain protein hydrolysates

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# THE FUNCTIONAL ORGANIZATION OF THE CEREBRAL CORTEX

WARREN S McCULLOCH

*Department of Psychiatry, University of Illinois College of Medicine, at the Illinois Neuropsychiatric Institute*

Functional organization, which defines the temporal course of activity in any nervous mechanism, depends upon both physico-chemical reactions of constituents and their anatomical relations Since reactions of all neurons are similar, it is frequently possible to deduce anatomy from observed activity or to predict activity from known anatomy

General organization From a single enlargement of the neural tube, the prosencephalon, arise the cortex cerebri and the corpus striatum anteriorly and the thalamus posteriorly At a comparatively early stage of development antero-posterior and postero-anterior connections are established They serve to relate certain portions of the cortex and basal ganglia (1, 2) to particular thalamic nuclei—which relay signals thither and receive them thence Also at an early stage, there emerge from the cortex axons which, passing ventrally, make their way to efferent structures Thus, functionally, these forebrain structures constitute a bridge of internuncials which in lower animals is but one of many As the cortex evolves, the other bridges either disappear or become unable to mediate all sense modalities This development correlates with increasing anatomic differentiation of the thalamic nuclei, which have few or no internuncials and, with few and small exceptions, no descending connections Hence, as the forebrain structures become necessary for afferents of a given sense modality to affect efferent systems, the cortical or striatal link also becomes necessary, and that in two ways It is both the internuncial system co-ordinating activity within and among the thalamic nuclei and the only significant path thence to the efferent system

The obvious paralyses resulting from injuries of certain cortical regions and the motor responses to their electrical stimulation have led us to call them "motor" or "electrically excitable", but today we know that most, if not all, regions of the cortex send axons to structures whose excitation affects the contraction of striated muscle, and that all parts of the cortex are electrically excitable Today we would not use these properties in subdividing the cortex The thalamic nuclear connections are discrete and serve to define "cortical sectors" consisting of cytoarchitectonically recognizable areas which preserve their topological relations—i e , those which are not altered by stretching or folding—presumably because the formation of these thalamo-cortical connections is causally related to the local differentiation of the cortex, and because they remain despite the growth and deformation of the continuous mantle as it evolves into the convoluted cortex Moreover, certain thalamic nuclei relay, *in an* orderly fashion, impulses consequent upon specific sensory excitation, and the corresponding cortical sectors exhibit this specificity and orderliness which help to define the principal rôle played by any sector and to analyze its spatial detail

in relation to that of the sense organs represented upon it. Even with respect to the associational structures which become disproportionately larger in the primate cortex and have thalamic relations with nuclear structures other than primary sensory relays—namely, parts of elaborate re-entrant circuits—the same clarification of function occurs for the same reason. Thus by conscientiously ignoring the obvious deformations, gyri and sulci, save as dubious landmarks in a particular cortex, it is possible to discuss in general the function of sectors and their constituent areas. Although the function of specific subdivisions of the sectors in other ways is various in various species, the functional homologies of these other subdivisions, insofar as they have corresponding thalamic correlates, are surprisingly orderly. This makes it possible to transfer to the cortex of a given species conclusions drawn from experiments upon other species. Since we are primates and most work has been done on the monkey *Macaca mulatta*, it seems best to use figures of his cortex as a frame of reference for what follows.

A cursory glance at the gross anatomy of any mammalian brain shows that the cortex on which a thalamic nucleus projects is many times larger than that nucleus, and detailed microscopy reveals that there is great overlapping of the projections. A single thalamic nuclear element thus projects to a vastly greater cortical area. As will appear clearly later, the connections in the reverse direction spread in a similar fashion. Thus the area of cortex to which a single thalamic neuron projects serves to relate it to a large portion of its own nucleus. On this account the nucleus and its cortical sector tend to behave concertedly in a rhythm determined by the time of transit around their reentrant path. At any part of the circuit the resultant waves affect the threshold to incoming stimuli and may be initiated or interrupted by them. Whether these waves be the envelopes of nervous impulses or merely of polarization and depolarization, they are prevented by any lesion which prevents corticothalamic reverberation. Residual activity lacks their characteristic form and frequency. Other deeper structures may contribute to the background of the thalamic excitation, and their contributions may be necessary, but the 'spontaneous' electrical activity will persist in a small island of cortex when all the rest of the cortex of that sector has been removed (3)—a finding which explains why diffuse pickup fails to reveal local cortical destruction.

It goes almost without saying that these same circuits serve as internuncials to relate the activities of diverse portions of the cortical sector. That these cortico-thalamo-cortical circuits play a fundamental role in determining the organization of cortical activity is attested by the synchronizing of disturbances at two parts of the same cortical sector after severance of all direct cortico-cortical connections. A similar but more complicated re-entrant path arising from cortical efferent connections serves a similar function for even larger subdivisions of the cortex. Thus, cortico-ponto-cerebello-thalamo-cortical circuits, arising from frontal, parietal and temporal regions and playing upon the anterior portions of the ventral thalamic nuclei, affect the activity of the anterior portion of the central, or sensory, sector.

This general character of overlapping projection extends to the control of

motion and makes possible use of parts whose focal cortical representation has been destroyed If the lesion be made early enough (4), although it be large, the loss of motion is far from total and investigation of the cortex by electrical stimulation indicates that other portions of the same cortical sector have far more control of the affected muscles than can be demonstrated in the intact cortex or following a similar lesion in the adult However, no sector has ever been shown to assume a function to which it did not contribute prior to the lesion The corpus striatum acts in parallel with the cortex and hence complete decortication leaves a well established thalamo-striatal prosencephalic bridge of internuncials, over which afferent are connected to efferent systems so that even in primates certain motor activities may still be mediated in response to stimulation of some sense organs

Relatively diffuse but overlapping projections upon the cortex where summation results in focal excitation make possible detailed discrimination, exceeding in sharpness of outline the distribution of excitation of sense organs (5), while interconnection of these cortical foci (6) via recursive connection through deeper structures and via direct cortico-cortical paths permits the activity of each focus at any instant to be affected by the antecedent and approximately contemporaneous activity of all other foci The resultant efferent discharges are thus the unified consequence of the totality of these related cortical focal activities Since activities of all parts of the cortex are related by recursive activities through deeper structures, severance of purely cortico-cortical connections can be expected to destroy only the most nearly contemporaneous relations of cortical activities at the separated foci, and so to affect adversely interpretations dependent upon them As yet we have no behavioral test whereby we might hope to detect them This is borne out by the lack of any discoverable loss of ability following severance of the entire corpus callosum, for this is the largest single bundle of such connections and the only one severable without any cortical destruction (7, 8)

On the other hand, overactivity of cortico-cortical connections produces a spread of disturbances over the cortex along these lines of communication This is all too familiar in the spread of the cortical activity responsible for the march of the Jacksonian convulsion, and today there is clear evidence that for this spread of disturbance direct cortico-cortical connections are necessary (9) and sufficient (10)

Common properties Before specifying particular thalamo-cortical projections or cortico-cortical connections, common properties should be considered, for these determine in large measure the significance of the particulars, and the interpretation of electrical or other records of cortical activity and its consequences

Impulses entering from the subjacent white matter reach all parts of the cortex via axons which ascend to its middle layer, where they branch widely and ramify as they ascend This axonal activity is manifest electrically by the appearance of a transient voltage in which the depth becomes negative and the surface positive to any unaffected area This phenomenon is not prevented by rendering the neurons of the recipient cortex unresponsive by local treatment with

drugs Having ramified, the incoming disturbance excites neurons of the superficial layers, producing a local surface negativity Under light barbiturate anesthesia this may be all that happens Under chloralose anesthesia and sometimes under very light barbiturate anesthesia, this surface-negative wave is propagated slowly—say 20 cm per second—in the feltwork of the cortex, dying out (11) as it travels Under appropriate conditions it can be elicited by weak electrical stimulation of these layers, and has been called the 'superficial response" Under extremely light anesthesia, or when augmented by dilute solutions of excitants such as strychnine or metrazol the surface-negative, or superficial, response is followed by a surface-positive, or deep, response associated with discharge of cells in the deeper layers of the cortex, whence originate axons to remote portions of the cortex, as well as those to subcortical structures These waves can be elicited regularly by stronger electrical stimulation and are conducted with axonal velocities There are, then, two types of purely corticocortical connections relating foci, at each of which arriving disturbances ascend to the middle layer, ramify upward and descend to leave the cortex The first is the slow, superficial, surface-negative, presumably multineuronal, or multisynaptic, intracortical type, exaggeratedly active under chloralose, the second is the rapid deep, surface-positive, presumably axonal, intercortical type, best seen under dial The intracortical are as diffuse as the feltwork of the cortex whereas the intercortical are highly specific and restricted in their distribution Under chloralose both play significant rôles in determining the spread of electrical after-discharge, under dial, only the latter (11) These types differ also in their effect upon incoming stimuli, for impulses arriving during surface negativity fail to excite, whereas those arriving during surface positivity are facilitated,—a relation which throws some light on the effect that alpha rhythm has upon response to stimulation of the eye or of the lateral geniculate and on the driving of alpha rhythm by visual stimulation (12, 13) Taken in conjunction with inhibition at a synapse in the case of the two neuron arc of the spinal cord (14), the relation of transcortical potentials to responsiveness to incoming stimuli suggests that inhibition is to be expected when the apical dendrite of any cell is in a region negative to its axon hillock, and facilitation when polarization is the reverse

Weak electrical stimulation of the superficial layers of the cortex, if repeated at appropriate intervals, elicits a depth response which increases to a certain maximum determined by the duration and voltage of the stimuli This facilitation occurs at all cortical foci and is manifest in motor response from many areas It is presumably due to spatial summation operative over relay paths and reverberating chains of internuncials That it has a maximum dependent upon other parameters of stimulation is presumably due to the same limitation of responding efferent cells that produces occlusion in the case of spinal reflexes with this difference that in the case of spinal reflexes all cells of the pool may be excited, whereas in the case of the cortex the number that may be called into play depends upon the voltage and duration of the stimulus Increase in voltage can always spread the stimulus to more remote portions of the pool and increase

in duration of pulse will, within limits, always fire a larger proportion of the neuronal pool reached by a given voltage Finally, repetitive activity at frequencies of 20 or more per second eventually produces a rise in threshold of the responding neurons, so that if the stimulation be continued the response declines or even disappears This rise in threshold is presumably the chief factor for the extinction of motor response to cortical stimulation, and undoubtedly is of paramount importance in all problems of fatigue It is associated with the development of a polarization of the correct sign to enhance the effect, and perhaps to extend it to other neurons in the vicinity In those cases in which stimulation of any cortical area is pushed so far as to initiate self-sustained activity—called after-discharge—another factor becomes significant, for the oxygen supply, though it may increase, is locally inadequate for the demand,

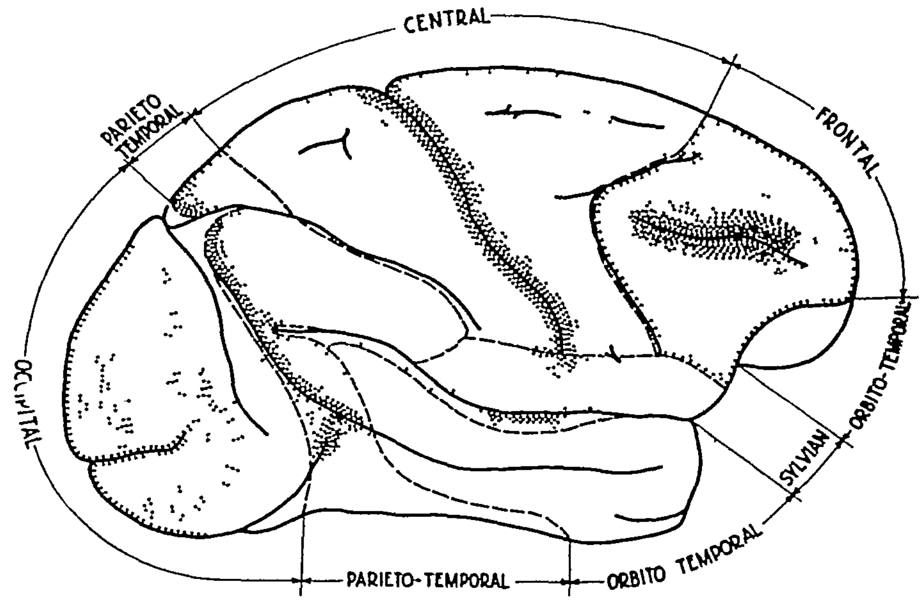


Fig 1 *Macaca mulatta* Thalamic projections to cerebral cortex and division into regions described in text

the oxygen tension falls (15) and lactic acid is produced (16) As the region becomes acid its threshold rises and eventually the self-sustained activity ceases It may have spread over cortico-cortical connections to other regions of the cortex, but those it leaves behind are inactive and unresponsive These are the aspects of functional organization which all parts of the cortex have in common

Cortical sectors and regions distinguished Thanks in no small measure to Walker's (17) and Le Gros Clark's (18) studies of retrograde degeneration of thalamic nuclei following destruction of various cortical regions, the "sectors" of the monkey's cortex defined by their thalamo-cortical connections are relatively well known They are schematized roughly in figure 1 which also indicates the density of thalamo-cortical projection by the density of stippling It is apparent that certain regions are dominated by these projections which define the *cortical sectors*

The projection from the lateral geniculate body in primates is sharply limited to area 17, the area striata, which is surrounded by area 18, and this, in turn, by area 19, known respectively as parastriate and preoccipital. The occipital region, constituted of these three areas, has a close functional organization subserving vision.

Similarly, there is a parieto-temporal region dominated by projections from the medial pulvinar nucleus to the posterior parietal cortex and by the inferior pulvinar nucleus to the posterior temporal cortex. The whole region plays an important rôle in associative processes.

The largest single region is the central or "sensory" region, which receives impulses from the lateral thalamic nuclei. Those anterior to the central sulcus come from the ventrolateral and those posterior from the posterolateral. The former are most dense in area 4, the latter in area 3, but as all parts of this region receive some, this sector is coextensive with this region.

Anterior to it is the frontal region whose thalamic projections arise from the dorso-medial nucleus and are most dense in a relatively small area which Walker has called area 46 for cytoarchitectural reasons. This region is granular cortex and includes all cortex anterior to the central sector on the lateral aspect, with the possible exception of area 8 which will be included in it to simplify description. On the medial side it extends just across the sulcus callosomarginalis. It does *not include the opercular areas or any orbital areas proper. The latter belong* to the orbitotemporal region.

The former, opercular, areas belong to the sylvian region which is dominated by the projection of the medial geniculate body to area 41 and with less density to area 42, known respectively as the primary and secondary auditory cortex and lying on the supratemporal plane which is therefore referred to as the sector of the medial geniculate body.

The sector of the anterior thalamic nucleus lies on the posterior portion of the medial aspect of the cortex and is coextensive with the posterior limbic region, consisting of areas 23 and a small retrosplenial area which is probably best called area 29.

The thalamic projections to the remainder of the iso-cortex are unknown. This includes the anterior limbic region, or area 24, and the large orbito-temporal region which includes all the areas confined to the orbital surface of the frontal pole and the tip and greater part of the lateral aspect of the temporal lobe. These are here regarded as one region, not because there are no known thalamic projections to them but because they are related in activity. Of the remaining isocortical area, 25—and of the allocortical areas 28 and 30—we lack the knowledge to assign them to any region.

Cortical sections and regions described. Among the regions of the isocortex there are three having sectors whose activity represents sensory excitation mediated by thalamic nuclei in such a manner as to preserve topological organization—namely the occipital, the central and the sylvian. These have been known for a relatively long time because lesions of these areas produced loss of the corresponding sensation or because excitation of the area, either in the aura of convulsive disorders or in the waking subject stimulated at operation,

produced the corresponding sensation More recently they have been investigated by stimulation of the receptor (19) and observation of the induced electrical disturbance

OCCIPITAL REGION By fixing the eye and flashing a small light in known relation to the point of fixation, while recording from electrodes in fixed positions on the occipital lobe, the visual field has been plotted on the cortex In the primate, off and on responses are sharply restricted (20) to area 17 The point of fixation is represented on its lateral or anterior margin and only one half of the field falls on one hemisphere Small angular displacements from the fixation point record at relatively large distances from its representation, but as the light is presented at angles increasing in equal steps the displacement per step is less on the cortex The horizontal meridian of the visual field maps almost horizontally backward from a point about the height of the junction of the first temporal sulcus and the sylvian fissure, in the direction of the superior ramus of the calcarine fissure The visual field represented is contralateral and inverted It is on this area to which each retinal point projects to an appreciable circle that summation results in disproportionately sharp differentiation, so that fine eye movements combined with this summation result in discriminative ability far exceeding the analysis of the stimulus of which the retina itself is capable (5)

To investigate the connections of various cortical foci, it is possible to stimulate at one focus and record induced electrical activity propagated to remote foci Electrical and chemical stimuli work, but the former are more difficult to control, for the current spreads to deeper structures where it excites subjacent axons, while it sets up antidromic impulses even in the gray matter These effects are minimized by using relatively long pulses at relatively low voltages, but they cannot be entirely excluded even under optimal conditions Hence the bulk of the work has been done with chemical stimulation If one places a square millimeter of filter paper soaked in a saturated solution of strychnine sulfate anywhere on the cortex, the cells of the subjacent cortex send out impulses in unison The resultant spike-like record of the transient voltage can be obtained from any point to which a sufficient number of affected cells send their axons For brevity, we say that the strychninized focus "fires" these other points At present there is reason to believe that strychnine poisons an acetylcholine esterase (21) and that this permits acetylcholine to accumulate, whence the cells are discharged so easily that the electrical impulse of any cell excites all the strychninized cells In any case strychnine acts only where synapses are present on nerve cells and produces disturbances propagated only in the ordinary direction of conduction

Local strychninization within area 17 produces these spikes but they are not propagated to points of area 17 more than about 1 mm from the strychninized focus At the same time there appear large spikes in the record of that portion of area 18 which is nearest to the focus We say, therefore, that area 17 fires itself only "locally," and fires area 18 Similar strychninization within area 18 fires an adjacent sector of area 17, fires almost all parts of area 18 of that hemisphere and the corresponding point in area 18 of the opposite hemisphere—and

fires much of area 19 ipsilaterally. Thus area 17, which receives impulses in a relatively discrete fashion, keeps them so within itself but relays them to a larger fraction of area 18, which feeds excitation back into the originating segment of 17 and forward into area 19. With a relatively light Dial narcosis one frequently obtains, following both "on" and "off" effects produced by a bright light, a series of smooth, somewhat sinusoidal waves. The "on" and "off" effects are limited to area 17, but the consequent waves spread forward across area 18 to die out in area 19. Moreover, electrical stimulation of any of these areas under appropriate conditions causes deviation of the eyes in a direction and manner dependent upon the exact site of stimulation. With ordinary 60 cycle currents one obtains from stimulation of any point in area 17 a sustained deviation which would bring foveal vision to bear on the corresponding point in the visual field (22). This form of stimulation is less effective in area 18 and the response is transient. Using pulses of rapid rising and slow falling phases, and a frequency up to about 40 per second, responses are greater and more prolonged but still not sustained. Their direction corresponds to that of the sector of area 17 to which the selected focus of 18 corresponds. Even slight stimulation of area 19 produces a relaxation of any existing muscular contraction and, in the vicinity of the intraparietal sulcus, pupillary dilatation. Moreover, stimulation of 19 will hold in abeyance the motor after-discharge, but not the cortical electrical after-discharge, set up by antecedent stimulation of other cortical foci. Finally, stimulation of this region causes a suppression of motor response to stimulation of any motor focus. This phenomenon has a latency of about four minutes, lasts from three to thirty minutes, and cannot be repeated with any certainty sooner than forty five minutes from the time of the stimulation that induced it. For brevity all three of these induced motor inactivities will be referred to as "suppression of motor functions." There is much evidence that they do not depend on any direct or indirect cortico-cortical connections or on cortico-striatal connections, but there is no certainty as to what descending paths mediate them. In these three suppressions cortex (23) and corpus striatum (24) act in parallel fashion.

Strychninization of area 19 causes only local firing and, with a variable latency of many minutes, a diminution or complete disappearance of spontaneous electrical activity which recedes slowly across the cortex, requiring twenty to forty minutes to reach the most remote regions. Before it has reached them activity returns to the nearer regions, at first in the form of "spindles" and later in its original form.

This suppression of electrical activity, like the suppression of motor function, can be elicited from several areas—which are therefore called suppressor areas. All such areas, except 19, have been shown to project to the nucleus caudatus, and for the suppression of electrical activity by them the nucleus caudatus has been shown to be necessary. Its strychninization produces large, long voltages in the thalamic nuclei which may well break up their cortical reverberations. These presumably are not simple axonal impulses from the nucleus caudatus because of time relations and wave form. They resemble the belated and extended post-synaptic consequences of the relatively synchronous discharges of

the pre-synaptic axons In any case, when they appear in a thalamic nucleus the "spontaneous" activity of the corresponding cortical sector disappears Until recently no trace of cortico-striatal activity could be discovered arising in area 19, but recently we have seen bumps in records from the nucleus caudatus which were definitely synchronous with strychnine spikes in area 19 Thus it, like all other suppressor areas, probably does produce its suppression of electrical activity via the nucleus caudatus We will consider the extrinsic cortico-cortical connections of this region only after the intrinsic regional connections of other regions have been considered

CENTRAL REGION The central sector has been mapped for its most direct sensory projection by punctate stimulation exciting single points of the body surface (25) or single hairs, and locating the induced surface-positive waves on the cerebral cortex The excited points project principally to the posterior lip of the central sulcus The representation of the extremities occupies a disproportionately large portion of the recipient cortex The leg is represented above a line joining the superior precentral sulcus to the superior postcentral sulcus The arm is represented below this line to another joining the spur of the arcuate sulcus to the tip of the intraparietal sulcus Although there is much overlapping in each of these subdivisions, the following general statements can be made The leg subdivision begins at the sulcus callosomarginalis, which represents the tail, coccygeal segments 4 through 3, then sacral segments 3 through 1, then lumbar segments 7, 6, 5, 4, and finally, lumbar 3 through 1, overlapped by thoracic segments 12 through 1 which appear at the line of junction of the arm and leg subdivisions Then come the cervical segments (again overlapping) in the order 2, 3, 4, 5, 6, 7, and last 8, which occupies almost the lower half of the arm subdivision The face area below is more complicated, for the mandibular division of the 5th nerve projects above, behind and below the other two, of which the ophthalmic is above and the maxillary below As the thalamic nuclei mediating sensibility from face, arm and leg are anatomically distinct and have been identified functionally, it is possible to subdivide the central sector along the lines described above, and to delimit these subdivisions in all primates The anterior lip of the central sulcus receives the densest projection of the anterior portions of these same thalamic nuclei, and the motor responses elicitable by its electrical stimulation are of the somatic parts projecting to the corresponding post-central area, except at the junctions of the subdivisions where the cortex of the anterior lip is most deeply infolded Thus the subdivision obtains for efferent as well as for afferent connections Actually, alteration of somatic muscular contraction can be obtained from all parts of this sector under appropriate conditions, and sensory phenomena can be elicited by strychninization of any part of it, and all exhibit the same subdivisions for leg, arm and face The conditions for obtaining motor response from points far posterior to the precentral lip are a hyperactivity of deeper structures, frequently appearing under light barbiturate anesthesia, and stimulating pulses having an abrupt rising and a 5 to 10 millisecond falling phase at a frequency of 1 to even 10 or more per second, delivered to a cortex already excited at a focus from which motor re-

sponses have been easily evoked The response is then that elicited from the original focus This form of so-called secondary facilitation, at least in certain instances, depends upon axons descending from both areas to the same deeper structures, rather than upon cortico-cortical connections (26) There is another form of secondary facilitation most easily demonstrated from points in the anterior portion of the central sector to points of the anterior lip of the central sulcus Antecedent stimulation of the anterior point makes it possible to obtain the original response to excitation of the posterior point with a weaker stimulus, or to obtain a larger response with the same stimulus This form of secondary facilitation is prevented by severance of cortico-cortical connections, and hence they presumably play some rôle in it

Strychninization of a few square millimeters within any subdivision of the central sector, performed under brief anesthesia from which the animal recovers before the effects of firing of the cortex have ceased, results in the appearance of all the familiar clinical signs of paresthesia and paralgesia in the apical portions of both sides of the body belonging to the subdivision strychninized (27) The reason for the bilaterality of the reference is by no means clear, for the afferent impulses are only contralateral from the arm and leg to the thalamic nuclei and to the cortex, and the symptoms are bilateral even when the strychnine is placed on an area practically devoid of callosal connections, so that neither cortico-thalamic nor cortico-cortical connections seem adequate to account for the bilaterality of reference implicit in the overt behavior That these sensory symptoms are due to thalamic rather than to cortical excitation can be deduced from this Strychninization of a few square millimeters of the most anterior portion of leg or arm subdivision fires all of both subdivisions, but only the thalamic nucleus of the subdivision strychninized and the symptoms are referred to the corresponding members Moreover, strychninization of the thalamic nucleus in the cat is sufficient to produce the symptoms, even when the cortex has been removed It was on the basis of these symptoms of sensory excitation that Dusser de Barenne outlined the 'sensory cortex" and subdivided it according to the part of the body subserved—leg, arm or face It was his hypothesis that the symptoms were to be explained by projection from every part of any subdivision to all parts of its thalamic nucleus That hypothesis has been amply substantiated by recording the electrical activity of each thalamic nucleus during strychninization of each of the constituent areas of each subdivision His 'sensory cortex" consisted of all those areas which Brodmann numbered 1 through 7 in his map of the monkey's brain Two difficulties have arisen with respect to this sector The lower margin of the face subdivision has proved to be higher on the frontal and parietal operculum than was originally supposed, and the method of local strychninization and recording of resultant electrical activity, which he called physiological neuronography, has compelled distinctions between parts of the cortex histologically indistinguishable by present methods To meet these difficulties and to indicate areas which have been identified cytoarchitectonically or physiologically since Brodmann's maps, we made figure 2, a, b and c (Hereafter numerals will be used to designate areas indicated by them

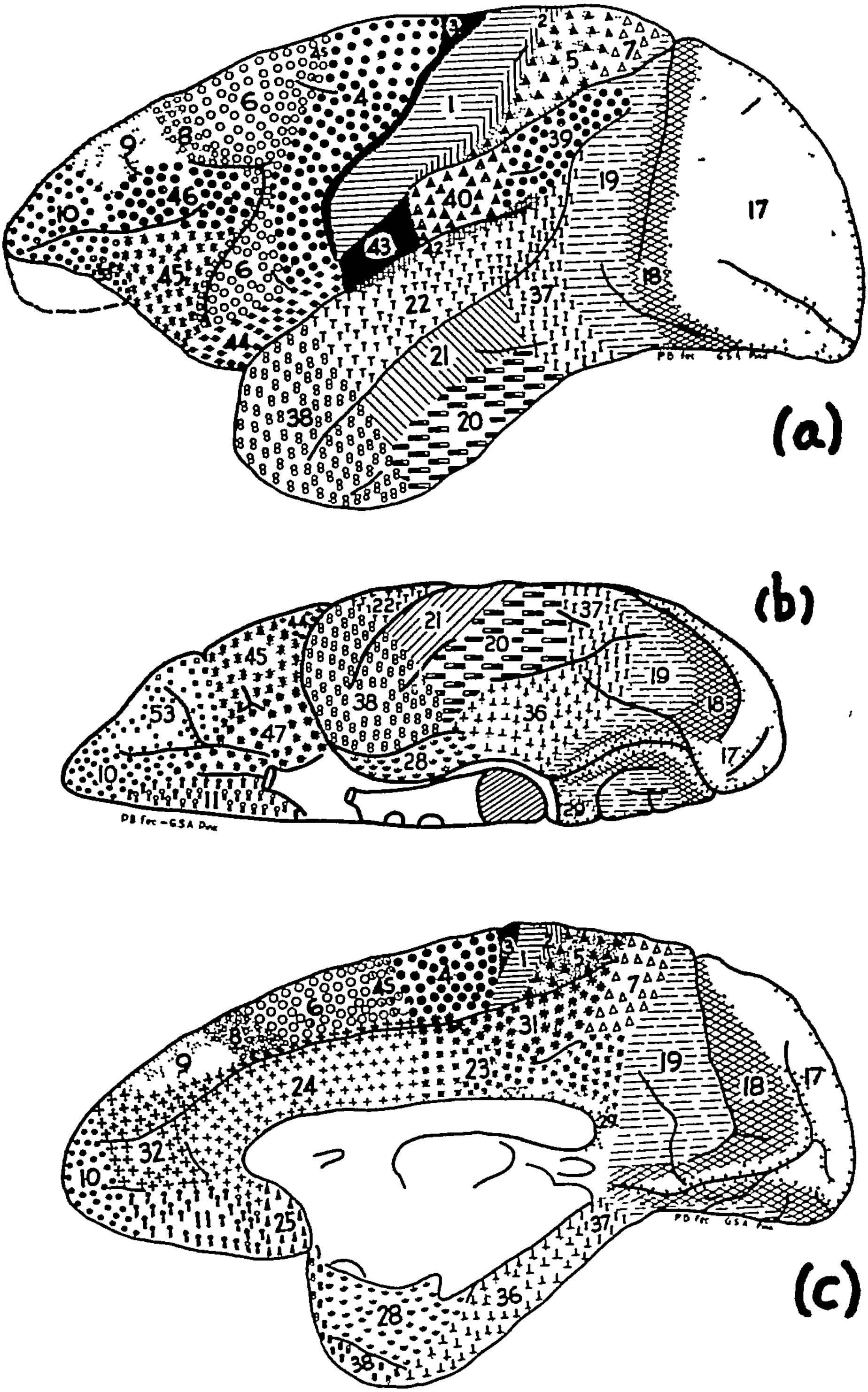


Fig 2 *Macaca mulatta* Areas of cortex distinguishable by cytoarchitectonics or physiological neuronography (a) lateral, (b) orbital and (c) medial surface

in this figure) The central sector, or "sensory cortex," consists of the areas 6, 4s, 4, 3, 1, 2, 5 In the monkey, 7 and both 40 and 39 (of whose cytoarchitectural identity with area 7 Brodmann was himself uncertain) are all sensory in Dusser de Barenne's sense of the term, and both fire the postero-lateral thalamic nuclei but poorly In the chimpanzee, 40 and 39 fail to fire it In both primates they fire heavily into the pulvinar Therefore, although their principal cortico-cortical connections are with the central sector, they must be considered as belonging to the parieto-temporal region, which is dominated by the sector of the pulvinar Excluding these and the opercular areas, the firing from one to another of the component areas can be stated briefly, 6, leg or arm, fires all component areas of both subdivisions, 4 fires itself, 1, 2 and 5, only in the face subdivision does it fire face 6, 1, arm and leg, each fires itself locally, and the corresponding 4 and 5 well, whereas 1 face fires 6 face and 4 face, 5, arm and leg, each fires itself more widely, and the corresponding 4, 1, 2 and 7, 4s and 2 fire themselves locally and suppress the electrical activity of the cortex generally They also suppress motor activity To this region neither 44 nor 43 properly belong, although there is evidence that taste may be projected upon 43 (28) and although motion of the tongue and larynx are obtained from 44 by primary stimulation and from 43 by secondary facilitation

SYLVIAN REGION For descriptive purposes, 43 and 44, although firing face 6, are considered part of the sylvian region, which is dominated by projection from the medial geniculate body to a slight elevation, corresponding to Heschl's gyrus, running from near the middle of the lower lip of the sylvian fissure diagonally upward and inward This corresponds to the primary auditory cortex of the cat, upon which the organ of Corti maps point for point The arrangement is exactly what is to be expected when the twist of the region from cat to monkey is kept in mind (29) Lower tones are represented on its anterior or lateral end, and higher tones more posteriorly or medially Still farther posteriorly, extending to the end of the sylvian fissure and sometimes emerging into the parietal region in positions corresponding to Campbell's "audito-psychic" area in the human brain, is what is probably best called the secondary auditory cortex, resembling the corresponding area in the cat The "primary" area, 41, hidden in the sylvian fissure, is koniocortex, and the "secondary," 42, the para-acoustic area contains very large pyramidal cells in layer III These areas are difficult to expose Even subpial resection of the parietal operculum leaves them in a condition which makes truly local strychninization difficult At present it seems best to confine remarks to the following statements 41 fires 42 42 fires itself well, and fires 41 as well as adjacent portions of 22 The posterior part of the superior temporal convolution fires area 42, but it is uncertain whether this is referable to strychninization of 22 or 37 No definite relation to any of the posterior parietal areas has been established, although in the cat partial asphyxia will cause a response to a click to appear in them synchronously with the response of the auditory cortex The requisite opercular destruction prevents study of the connections of one to the other beneath the sylvian fissure except from the surfaces exposed in the intact cortex These have shown the following connec-

tions 43 fires 44 and 22, 22 fires 44 and 43 and probably 42 Thus this region contains structures necessary for vocal response to auditory stimulation, and their cortico-cortical connections are direct

PARIETO-TEMPORAL REGION The region lying between the occipital posteriorly and the central and sylvian anteriorly, is roughly triangular with an extension forward on the medial aspect, whence it extends to a wide base along the inferior aspect of the temporal lobe It includes 31 and probably 7 on the medial aspect, and 39, 40, 37, 20 on the convexity of the hemisphere It is dominated by areas 39 and 37 which receive the bulk of the pulvinar projections Each of its constituent areas fires itself well but its inter-areal connections are relatively few or restricted 31 fires none of the other constituents The line between 39 and 40 is vague, and these areas may fire each other, but only from neighboring portions The upper limit of 37 is equally vague, and 37 fires 39 Similarly, more work is necessary to bound 37 anteriorly, but there is nothing to suggest that it fires or is fired by areas anterior to it Per contra, its inter-regional relations are obvious and important, as will appear later

LIMBIC REGIONS The posterior limbic region, or sector of the anterior nucleus (30), lying between 31 and the corpus callosum, is made of two areas one, which fires only itself and that only locally, called 29 for histological reasons, the other, 23, which fires itself throughout The anterior limbic region is a single suppressor area, 24, which, like other suppressor areas, fires itself and only locally Of areas near its anterior end—i e , 25 and 11, we lack information and hence are unable as yet to assign them to this region or to the orbito-temporal or frontal regions

FRONTAL REGION The frontal region is bounded posteriorly by the central, medially by the anterior limbic and orbitally by 11, 47 and 53 In the monkey this region has not been clearly analyzable by local strychninization and distribution of firing within the region, except for two areas 32, which fires itself throughout but no other frontal area, and 8, which is a suppressor area and fires itself extremely locally and fires into 32 Moreover, area 8 is "motor" for contralateral, and anterosuperiorly for ipsilateral, eye movements associated with dilatation of the pupil This dilatation, in the case of the cat, is due to inhibition of the third nerve nucleus (31) As of eye movements obtained from 17, 18 and 19, it is possible to conclude that the cells of origin for the requisite structure lie in the area in question Eye movements are obtainable from the parietal cortex generally, if short, high voltage pulses are used These can stimulate underlying white matter, and thermocoagulation of the parietal cortex does not prevent them from being elicited, whereas it abolishes those evoked from 8 Unilateral destruction of 8 produces a pseudo-hemianopia (32) and circus movements Immediately anterior to its central third lies 46, which receives the densest projection from the dorso-medial nucleus Its strychninization results in relatively wide firing of the frontal pole Anterior to it is 10 where firing is most restricted The cortex medial, or dorsal, to area 46, being bounded by areas that can be thus defined, is called 9 The area ventral, or lateral, to 46—i e , 45, is distinguished by its firing 44 and 40

ORBITO-TEMPORAL REGION The remainder of the isocortex, characterized by deficit of thalamic connections, constitutes the orbito-temporal region The orbital areas, 47 and 53, are easily distinguishable, for electrical stimulation of 47, but not of 53, causes an arrest of respiration in inspiration with the vocal cords abducted—as in a yawn Moreover, they fail to fire each other and are cyto-architecturally distinct, for 47 is dysgranular and 53 is eugranular The temporal areas 38, 21 and 22 are difficult to distinguish histologically in the monkey Each fires itself throughout but fails to fire the others The relation of the orbital to the temporal components is as follows 47 fires 38, 53 fires 37, 38 fires 47, 22 fires 53 It seems safe to assume that these connections form the bulk of the fasciculus uncinatus

INTRACORTICAL CONNECTIONS While we have been considering only the inter-cortical connections as revealed by strychninization under dial narcosis, it must be remembered that the belt of intra-cortical fibers exists and under other conditions can be shown to relate relatively distant foci As these connections run in all directions, they relate areas regardless of the regions or sectors to which they belong They cannot, however, relate an area to a distant one without crossing those areas which surround the excited area This is strictly a topological problem in two dimensions It is, therefore, invariant under continuous deformation of the cortex Hence it is possible to map the entire cortex and the basal ganglia on a plane without losing the relations determined by the intra cortical connections Figure 3 is such a map In it the margins of the cortex of one hemisphere appear stretched around the periphery, and the adjacent areas are consequently greatly elongated Moreover, all the sulci are treated as if they were opened out This, for example, discloses area 3 and the continuity of area 6 from arm to face subdivisions, and brings to the surface the insular cortex In so doing it makes clearer the lack of information The basal ganglia have been so displaced as to make them visible without severing their continuity with cortical structures either anteriorly, where 47 is continuous with the putamen, or posteriorly, where the amygdaloid nucleus adjoins the allocortex of the temporal lobe

INTER REGIONAL CONNECTIONS The connections between the occipital and parieto-temporal regions probably constitute both the fasciculus longitudinalis inferior and the fasciculus occipitalis verticalis (of Wernicke), and the long antero-posterior connections, the superior longitudinal fasciculus Just at the time that the connection of all suppressor areas to the belt-like areas 31, 32, were discovered by physiological neuronography, the connection of 4s to this same band was followed by myelin stain (33) Most of the remaining connections as yet lack correspondingly precise anatomical footing Since the corpus striatum not only arises in parallel with the cerebral cortex, but also acts in parallel with it, at least in suppression of motor activity, the cortico-striatal connections established by physiological means are here included Areas 24, 8, 4s, 2 and probably 19 all fire the nucleus caudatus, whereas 6 fires the putamen and the external segment of the globus pallidus, and 4 fires the putamen but not the external segment of the globus pallidus Recently by a new method applied

to the cat's brain, connections from some suppressor areas to the nucleus caudatus have been shown to be by fine nonmyelinated collaterals from descending axons (34) This accounts for previous failures to find them by Marchi studies of degeneration (35)

INTER-HEMISPHERICAL CONNECTIONS For the most extensive anatomical studies of the connections of the two hemispheres we are indebted to Mettler (36, 37, 38, 39), according to whom almost all parts of one hemisphere send

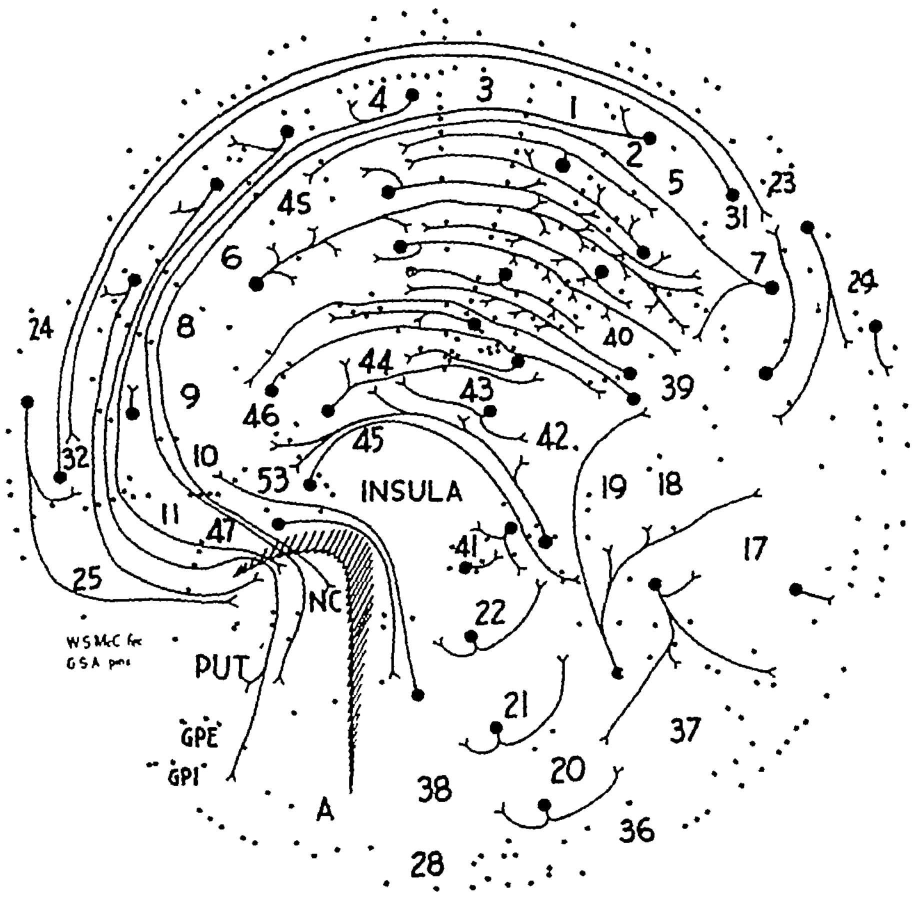


Fig 3 *Macaca mulatta* Development of cortex and basal ganglia preserving topological relations On it are indicated all cortico cortical and cortico striatal connections demonstrated by physiological neuronography

axons to a corresponding but larger and related portion of the other, while certain regions send them to many portions of the opposite hemisphere—so-called heterotopic connections Possibly because the lesions affected the underlying white matter, the projections described by him are more extensive than those found by electrical stimulation, although this may have excited certain of the more superficial fibers The reason for the difference may also be that the discovery of the electrical disturbance on the receiving hemisphere depends

necessarily upon the synchronous activity of enough axonal endings in a small space A diffuse projection might easily be missed This applies also to physiological neuronography, with this difference that the strychnine can excite only neurons, not axons or axonal terminations Hence its findings can only be of fewer projections than by these other means Figure 4 schematizes the origin of the commissural connections as revealed by physiological neuronography It discloses the direction as well as the most concentrated axonal distributions rather than their totality

Mettler used the term "homoiotopic" for the more diffuse anatomical projections to corresponding related portions of the contralateral hemisphere These are always restricted to constituent areas of the same contralateral region Even what he has called heterotopic" connections rarely exceed these limits

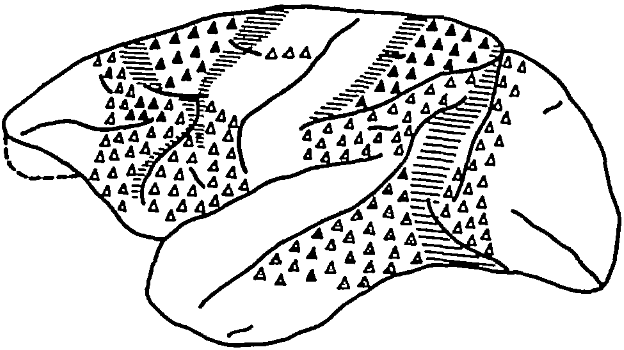

Fig 4 *Macaca mulatta* Cortical origins of commissural connections ▲ to widely separated points △ to symmetrical point only and stopped by section of corpus callosum △ to symmetrical point only but persisting though diminished after section of corpus callosum

When they do so the exceptions conform to the interregional connections of the homolateral projections There is an additional reason, therefore, for inquiring into the latter in some detail

REMAINING DIFFICULTIES In the first place, the study of the interregional connections has not been pushed as hard as the studies of the functional organization of the regions themselves, and there may be many connections as yet undiscovered, but the connections so far revealed are so numerous and intricate as to make any regional subdivision seem somewhat arbitrary This is particularly true with respect to areas 31 and 32, which constitute a narrow band along the sulcus calloso-marginalis, with extensions at its anterior and posterior extremities The extremities are clearly different, histologically, but in the intermediate region both areas shade into the adjacent areas of the central region so that no definite boundaries can be established Moreover, this band is

internally connected by axons from any part to all parts in the monkey, whereas in the chimpanzee the connections are by no means as strong to distant portions In the monkey, all parts of the band are fired from all suppressor areas, whereas in the chimpanzee the firing is more restricted To assign different portions of this band to different regions seems arbitrary in spite of the dissimilarity of thalamic connections A similar difficulty occurs with 7, 39 and 40, which are unmistakably sensory (as that term was used by Dusser de Barenne) Moreover, they have powerful connections to and from the rest of the central sector despite their projections to and from the pulvinar The entire parieto-temporal region is an extreme example of long, interregional connections, for there is no region on the convexity of the hemisphere with which it fails to make connections in both directions Inferentially, it subserves associational functions of a high order The frontal region obviously has not differentiated as far in the monkey as in the chimpanzee, nor has it as extensive connections with the rest of the hemisphere The sylvian region containing the projection areas for sound and the control of respiration, lingual and laryngeal musculature, and the interconnections of these areas, is obviously the precursor of those cortical structures which in man are principally responsible for speech Unfortunately, they are hidden in the sylvian fissure to such an extent that their connections are difficult to investigate, but it is clear that they are in part, at least, recipients of impulses from the portion of the temporoparietal region from which the angular and supramarginal gyri of man arise The behavioral implications are self-apparent, for there do not exist any connections in the thalamus whereby these areas could be related once the cortex is destroyed Comparison of the newest maps of interregional connection with antecedent studies indicates how easily these have been missed, and a recognition that that part of the cortex lying in sulci has never been investigated implies that many more are still to be expected Obviously, no conclusions can be drawn from the apparent lack of connections of particular regions Only one general statement concerning these connections seems to hold namely, that the primary sensory areas do not give rise to interregional associational fibers This again emphasizes the importance of the "associational areas" It is apparent today that certain tenets of the psychology of perception can be deduced from the physiology of the primary sensory areas In similar fashion we have every right to expect that utimately knowledge of "associational areas" and of interregional connections will lead to a physiological interpretation of the more complex psychological problems

In order to bring this article (which is essentially a review) as nearly up to date as possible, I have taken the liberty of including hitherto unpublished work done in collaboration with G von Bonin, H W Garol, E W Davis, A Silveira and P Bailey—to the last of whom we are indebted for figure 2

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PHYSIOLOGICAL REVIEWS

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# FACTORS AFFECTING THE INSULIN CONTENT OF THE PANCREAS

R. E HAIST

*Department of Physiology University of Toronto*

*The pancreas as the source of insulin* Insulin, the anti-diabetic hormone, is produced in the pancreas (1) and, despite the earlier literature to the contrary, there is no good evidence that it is produced in any other organ or tissue (2) The beta cells of the islets of Langerhans are conceded to be the secreting structures The demonstration that insulin can be obtained from the principal islets of teleost fishes and not from acinous tissue (3) and that it is found in metastatic islet cell tumors (4) indicates that the islets of Langerhans are responsible for the production of this material That the beta cells are the actual secreting elements seems likely from the fact that the severity of the diabetes in partially depancreatized dogs (5) (6) or in dogs injected with anterior pituitary extract (7) (8) (9) parallels the extent of the change in the beta cells of the islets Indeed, the degree of beta cell granulation demonstrated with Bowie's staining technique in the islets of pituitary-injected dogs (7) (8) (9) (10) and in the islets of fasting and fat-fed rats given insulin (11) shows a close correlation with the insulin content of the pancreas

*The significance of the insulin level* If it is granted that the beta cells of the islets of Langerhans in the pancreas are the insulin producing cells, then it will be apparent that the insulin content of the pancreas is dependent upon *1*, the *concentration* of insulin in the beta cells of the islets, and *2*, the total volume of the beta cells It follows then that some of the factors influencing the insulin content of the pancreas will bring about their effects by altering islet volume whereas others will change the concentration of insulin in the islet cells If the beta cell volume remains constant, at any moment the concentration of insulin in the pancreas will represent a balance between the production and liberation of that material Such a balance might be struck at any level, so some level-regulating mechanism must be postulated. Many of the same difficulties are involved in a discussion of the level of pancreatic insulin as in a consideration of the blood sugar level (12) or, for that matter, the level of any material in the body normally present in fairly constant amounts

At any level, the turnover of insulin might conceivably be great or small but so long as production and liberation keep pace the level will remain constant The fact that when only a fraction of the pancreas remains, the animal may be free from diabetes and that under these circumstances the pancreatic insulin concentration may be unchanged (13) (14) would indicate either that only a fraction of the pancreas is functioning under normal conditions or that there is a greater than normal turnover of insulin in the remnant without any great change in level Nothing is known of the mechanism which stimulates or diminishes production when the liberation of insulin is altered, yet some such regulation under a fairly wide range of conditions would seem to be a necessity

It should be emphasized, however, that the insulin level as measured by any of the present methods is sufficiently variable to make measurements of small changes in content uncertain and to make conclusions on the basis of such changes unwarranted Such small changes may be significantly concerned with the normal variation in production and liberation Studies on the partially depancreatized non-diabetic dog (13) (14) would indicate that the concentration or level of insulin in the beta cells is probably of more significance than the total content, since in these animals the total content may be drastically reduced (by operation) without greatly changing the level of insulin in the pancreas or altering the state of the animal

A lowering of the insulin content of the pancreas must result from either a reduction in the number of functioning beta cells or some disparity between the production and liberation of insulin The disparity may be caused by a reduction in the manufacture of insulin that is out of proportion to any change in liberation, or an increased liberation of insulin that is out of proportion to any change in production A reduction in the insulin content of the pancreas may result then from an increased liberation of insulin, a decreased production of insulin or both Once the new level is reached, it may be maintained by again balancing production and liberation What regulates turnover at any given level or operates to alter the level is the unknown but important factor It is obvious that a determination of the level of pancreatic insulin *per se* may tell little of what is happening in the pancreas Many determinations must be made under a variety of experimental conditions and correlated with associated metabolic and histological changes if the significance of the insulin level is to be understood

*Methods involved in the determination of the insulin content of pancreas* The estimation of the insulin content of the pancreas under most circumstances involves the removal of all the pancreas from the animal, the extraction of the tissue removed and the determination of the insulin content of the resultant solution The removal of the pancreas must be complete, and all the pancreatic tissue should be extracted This is particularly important in those animals having a pancreas that is diffusely distributed and may contain varying amounts of fat Also, since the concentration of insulin is not the same in all parts of the pancreas (14) a sample portion may give an erroneous impression of the insulin content

The extraction procedures have differed considerably A great deal of work has been done on this problem and its discussion is beyond the scope of this paper Perhaps the most commonly used extraction fluid is acid alcohol Much of the recent work employs Scott and Fisher's modification (16) of the procedure of Jephcott (15) It should be pointed out that in using this procedure the recovery of crude insulin added to the tissue is good, while the recovery of purified insulin is poor and variable (0–96 per cent) (15) Marks and Young (17) commented on their inability to recover all added insulin by the method of Scott and Fisher, but failed to note Jephcott's earlier observations Since crude insulin is recovered almost completely, the method would seem to be more satisfactory than the partial recovery of added purified insulin would indicate In tests made on

aliquots of two homogeneous preparations of pancreatic tissue obtained in each instance from 50 rats, Best, Haist and Ridout (23) (73) found this extraction procedure to give remarkably consistent results

The methods of measuring the insulin potency of the pancreatic extracts differ considerably in the different laboratories Both rabbit and mouse assay procedures have been employed but the latter have been used more commonly for the experimental investigations The "mouse" methods depend upon injecting the test solution into mice and noting the number of mice which convulse This is compared with the effect of a standard insulin solution Some workers use several dilutions of unknown and standard solutions and compare the relation of the two convulsion-dose curves (18) (19) Others use one dilution of standard and one of unknown that causes approximately 50 per cent of the mice to convulse A large number of mice are tested at this dilution and the relation of the potencies of unknown and standard are found by reference to a characteristic dose-response curve previously obtained on a large number of mice (20) (21) A comparison of the methods is given by Gaddum (22) The accuracy of either procedure is dependent upon the number of mice used The number used constitutes an important point of difference between the tests as carried out by different workers and is not often mentioned in their reports

*Manner of expressing results* The concentration of insulin in the beta cells of the islets is undoubtedly the important value to determine but since the simultaneous measurement of beta cell volume and insulin content of pancreas is not feasible, other methods of expressing the results must be followed The way in which the results are expressed differs in the various reports Some give the results as units of insulin per kilogram of body weight, others as units per gram or kilogram of pancreas and others as units per rat or group of rats None of the methods of presenting the results is satisfactory under all circumstances In those animals having a diffusely distributed pancreas, any expression on the basis of weight of pancreatic tissue becomes of doubtful value because variable amounts of fat and other material usually are present (23) In the rat, whose pancreas is diffuse, it is convenient to compare the total insulin content of the pancreas of the rats or groups of rats This method is of value only if the animals in control and test groups are as nearly similar as possible at the start of the experiment When a relatively homogeneous population of cases can be obtained, it is the method of choice Expressions on the basis of body weight are useful, though here too it is possible to fall into error Frequently there is no comment to indicate whether the reference is to the initial or final weight of the test animals The comparison of insulin contents based on the final body weight is sometimes misleading For example if, in the test group, the loss in body weight is greater than the reduction in insulin content and the results are expressed in terms of final body weight, then an increase over the control group will be indicated whereas no actual increase has occurred and the total content may be even less than in the controls Under varying conditions the insulin content of the pancreas need not bear any specific relationship to body weight (23), though it is true that in *normal untreated* rats

there is a rough relationship between insulin content and body weight (24) (25) In dogs, the population of cases is not homogeneous and here it is frequently more illuminating to indicate the *concentration* of insulin as well as the total amount of insulin in the pancreas

TABLE 1

*Insulin content of pancreas in different species*

| SOURCE | UNITS/GRAM PANCREAS | WORKER | METHOD | DATE |
|---|---|---|---|---|
| *Birds* | | | | |
| Chicken | 0 76 | Redenbaugh, Ivy and Koppanyi (31) | Alcohol $H_2SO_4$ | 1926 |
| Chicken | 0 65 | Jephcott (32) | Alcohol-HCl | 1931 |
| Duck | 0 53 | Jephcott (32) | Alcohol-HCl | 1931 |
| *Mammals* | | | | |
| Mouse | 1 7 | Marks and Young (26) | | 1940 |
| Rat (Wistar) | 2 1 | Best, Haist and Ridout (23) | Alcohol-HCl | 1939 |
| Rat (Wistar) | 0 6 | Soong (33) | Alcohol-HCl | 1940 |
| Rat (Wistar) | 1 3 | Marks and Young (26) | Alcohol-HCl | 1940 |
| Rat (Wistar) | 1 0 | Griffiths (24) | Alcohol-HCl | 1941 |
| Rabbit | 7 8 | Marks and Young (26) | Picrate-acetone | 1940 |
| Rabbit | 9 5 | Marks and Young (17) | Alcohol-HCl | 1940 |
| Rabbit | 5 2 | Griffiths (34) | Alcohol-HCl | 1942 |
| Cat | 2 0 | Baker, Dickens and Dodds (35) | Acetone-picric acid | 1924 |
| Cat | 0 68 | Takeuchi (36) | Acetone-picric acid | 1928 |
| Cat | 2 5 | Jephcott (32) | Alcohol-HCl | 1931 |
| Cat | 1 7 | Scott and Fisher (16) | Alcohol-HCl | 1938 |
| Cat | 2 2 | Marks and Young (26) | Alcohol-HCl | 1940 |
| Dog | 0 8 –1 5 | Nothmann (37) | Alcohol-$H_2SO_4$ | 1925 |
| Dog | 2 1 | Takeuchi (36) | Alcohol-$H_2SO_4$ | 1928 |
| Dog | 2 6 | Takeuchi (36) | Acetone-picric acid | 1928 |
| Dog | 3 8 | Jephcott (32) | Alcohol-HCl | 1931 |
| Dog | 5 0 –5 5 | Murray and Waters (38) | Alcohol-HCl | 1932 |
| Dog | 3 4 | Best, Campbell and Haist (7) | Alcohol-HCl | 1939 |
| Dog | 3 3 | Marks and Young (26) | Alcohol-HCl | 1940 |
| Guinea pig | 0 08 | Marks and Young (26) | Alcohol-HCl | 1940 |
| | 0 23 | Marks and Young (26) | Picrate-acetone | |
| Beef | 0 75–1 2 | Wernicke (39) | Alcohol-HCl | 1924 |
| Beef | 1 5 –2 5 | Somogyi, Doisy and Shaffer (40) | Alcohol-$H_2SO_4$ | 1924 |
| Beef | 0 9 –1 5 | Dudley and Starling (41) | Alcohol-$NaHCO_3$ | 1924 |

TABLE 1—*Concluded*

| SOURCE | UNITS/GRAM PANCREAS | WORKER | METHOD | DATE |
|---|---|---|---|---|
| *Mammals*—Cont'd | | | | |
| Beef | 2 5 | Baker, Dickens and Dodds (35) | Acetone-picric acid | 1924 |
| Beef | 4 4 | Moloney and Findley (42) | Alcohol | 1924 |
| Beef | 1 8 | Fenger and Wilson (43) | Alcohol HCl | 1924 |
| Beef | 2 0 | Langecker and Wiechowski (44) | Acid alcohol | 1925 |
| Beef | 1 8 -2 2 | Scott and Best (45) | Alcohol $H_2SO_4$ | 1925 |
| Beef | 3 3 | Scott (46) | Alcohol HCl | 1925 |
| Beef | 1 8 -2 5 | Blatherwick et al (47) | Alcohol $H_2SO_4$ | 1927 |
| Beef | 1 3 | Takeuchi (36) | Acetone picric acid | 1928 |
| Beef | 1 3 | Kaulbersz (48) | Alcohol $H_2SO_4$ | 1930 |
| Beef | 1 4 | Kaulbersz (48) | Alcohol $NaHCO_3$ | |
| Beef | 1 4 | Kaulbersz (48) | Water HCl | |
| Beef | 1 5 | | Water $NaHCO_3$ | |
| Beef | 3 0 -3 5 | Jephcott (15) | Alcohol HCl | 1931 |
| Beef (2 years) | 4 8 | Fisher and Scott (49) | Alcohol HCl | 1934 |
| Beef (9 years and older) | 1 8 | Fisher and Scott (49) | Alcohol HCl | 1934 |
| Pig | 1 6 -3 1 | Dodds and Dickens (50) | Water formic acid and formaldehyde | 1924 |
| Pig | 5 5 | Dodds and Dickens (50) | Acetone picric acid | 1024 |
| Pig | 3 0 | Clough Allen and Murlin (51) | Water HCl | 1924 |
| Pig | 3 0 -4 3 | Baker Dickens and Dodds (35) | Acetone picric acid | 1924 |
| Pig | 1 7 -1 9 | Fenger and Wilson (43) | Alcohol HCl | 1924 |
| Pig | 2 1 | Takeuchi (36) | Alcohol $H_2SO_4$ | 1928 |
| Pig | 1 3 | Takeuchi (36) | Acetone picric acid | 1928 |
| Pig | 2 0 | Jephcott (32) | Alcohol HCl | 1031 |
| Sheep | 1 0 | Baker, Dickens and Dodds (35) | Acetone picric acid | 1924 |
| Sheep | 1 8 | Fenger and Wilson (43) | Alcohol HCl | 1924 |
| Sheep | 0 7 | Jephcott (32) | Alcohol HCl | 1931 |
| Horse | 1 5 | Baker Dickens and Dodds (35) | Acetone-picric acid | 1924 |
| Horse | 1 4 | Takeuchi (36) | Acetone-picric acid | 1928 |
| Horse | 2 0 | Jephcott (32) | Alcohol HCl | 1931 |
| Monkey (Macacus rhesus) | 2 5 | Jephcott (32) | Alcohol HCl | 1931 |
| Chimpanzee | 11 2 | Marks and Young (26) | Alcohol HCl | 1940 |
| Man | 0 24 | Pollak (52) | | 1926 |
| Man | 0 84 | Takeuchi (36) | | 1928 |
| Man | 1 7 | Scott and Fisher (53) | Alcohol HCl | 1938 |

*Insulin content in normal animals of different species* The total insulin content of the pancreas obviously will vary greatly from species to species The concentration of insulin in the gland, however, is far more constant Normal values have been reported for the following fish, chicken, duck, mouse, rat, guinea pig, rabbit, cat, dog, cattle, pig, sheep, horse, monkey, chimpanzee and man The values obtained, the extraction method used, the references and dates are given in table 1 Some unpublished data obtained by C M Jephcott in 1931 are included in the table

It will be evident that the concentration of insulin in the pancreas of mammals is somewhat similar in the different species The values for the rabbit and chimpanzee are high, while those for the guinea pig are unexpectedly low (26) Judging from the duck and chicken, fowl have lower concentrations of insulin in their pancreases than most mammals The values in teleost fish, which are not included in the table but range from 2 to 13 units per gram, can hardly be compared with the others since only the principal islets were used for extraction and these are composed almost entirely of islet tissue (27) (28) (29) Using the $\frac{\text{islet} \times 100}{\text{acinar}}$ ratio of 1 1 obtained by Richardson and Young (30) for Wistar rats, and insulin values per gram of pancreas obtained by Marks and Young (17) and by Best, Haist and Ridout (23), it can be calculated that the insulin per gram of *islet* tissue for the Wistar rat is somewhere in the neighborhood of 119–193 units, a value much higher than any reported for the principal islets of fish The values for the insulin content per kilo of body weight of animal, though not given in the table, do not show a great range, indicating that in normal animals the total insulin content is roughly proportional to body weight Considerable variation in insulin concentration and total content have been reported for single species One important factor in these differences is the age of the animals

*Insulin content and age* The insulin content of the pancreas changes with age In the cow, the *concentration* of insulin in the pancreas decreases as the animals become older (49) Fetal calves under 5 months show an average of 33 2 units per gram, fetal calves 5 to 7 months, 23 1 units, calves 6 to 8 weeks old, 11 4 units, 2-year heifers, 4 8 units, cows 9 years and older, 1 8 units per gram of pancreas The *total insulin content* of the pancreas of Wistar rats, however, increases with age (24) (25) In normal humans above 12 years of age the pancreatic insulin does not seem to be affected by length of life (53) However, data on islet volume changes would indicate that during the first few years after birth the insulin content of human pancreas probably would show a substantial increase with age Ogilvie (54), in an intensive study of islet and acinar volumes and islet counts in the pancreases of 100 human subjects varying from birth to 64 years of age, found that the average islet weight per pancreas increased from 0 12 gram at birth to 1 07 grams at 21 years The weight of islet tissue varied greatly, however, in any age group There was a rapid increase in islet tissue at first, with a tendency to level off at the third year but with a rapid increase again during adolescence, and a final stabilization at about 21

years The total islet count increased from an average of 284,000 at birth to an average of 960,000 at 3 years, after which it remained relatively constant In the first few years of life therefore the increase in the number of islets seems to account for the increase in islet weight, whereas during childhood and adolescence the increase appears to be brought about by an increase in the size of the islets After 21 years of age the amount of islet tissue per kilogram of body weight is fairly constant, though after 45 years of age the increase in body weight is greater than that of islet tissue (54)

While in certain species, then, the *concentration* of insulin in the pancreas is higher in fetuses and very young animals, yet in others it would appear that the *total* insulin content of pancreas increases with age though it may tend to level off when adult life is reached

*Seasonal variation in insulin content* Fisher and Scott (49) found that better yields of insulin were obtained from beef pancreas during the winter months Nitzescu (55) obtained greater yields from June to September than from November to February However, other factors, such as age and the freshness of the tissue, which usually are not well controlled in studies on beef pancreas, may be involved in these variations The insulin content of pancreas does seem to fluctuate when observed over a period of time in carefully controlled experiments but the factors involved are not clear No seasonal relationship has been definitely established

*Distribution of insulin in the pancreas* The distribution of insulin in the pancreas of the dog is not uniform It was found that the free splenic end of the pancreas had the highest content of insulin (4.2 units per gram) The attached duodenal portion was next (3 1 units per gram) and the free duodenal end had the lowest concentration (2.2 units per gram) (14) Figures are not available concerning the distribution of insulin in the pancreas of other animals, but the above order of distribution was observed for islet counts in guinea pigs (56) In humans also the greatest number of islets was found in the splenic end of the pancreas (57) The data do not allow a good comparison of the distribution of insulin in the pancreases of different species There is sufficient evidence however, to indicate that the concentration of insulin is not uniform throughout the whole pancreas, a fact that must always be kept in mind when a part of the pancreas is taken for extraction

*Insulin content and anesthesia* Anesthesia with urethane and sodium amytal was found by Best, Haist and Ridout (23) to cause no appreciable alteration in the insulin content of pancreas as compared with the values obtained in stunned rats This was true despite the fact that the blood sugar was greatly elevated in the group receiving urethane The effect of other anesthetics has not been reported

*Ligation of ducts* Herxheimer (58) showed that extracts of duct-ligated pancreas were more effective in lowering blood sugar than extracts of normal glands Ligation of the ducts leads to atrophy of the pancreas as a whole though it has been reported to cause regeneration of the islets (59) (60) Atrophy of acinous tissue occurs more rapidly than that of the islets in duct ligated

pancreas The original islets, however, atrophy also and regeneration does not restore the islets to their normal state (61) This differential atrophy leads to an increase in the islet-acinar ratio and would account for an increased insulin *concentration* but a decrease rather than an increase in *total content* would be expected Such a reduction in the total insulin content of the pancreas has been noted by Waters and Best in dogs 8 to 10 weeks after ligation of the pancreatic ducts (personal communication)

*Infection* Menten and Krugh (62) were unable to determine any appreciable alteration in the insulin content of the rabbit pancreas following single intravenous sublethal doses of paratyphoid B filtrates, despite the fact that hydropic degeneration of islet cells may occur in rabbits and guinea pigs suffering from infection with enteritides-paratyphosus B group, and despite the fact that injections of paratyphoid B filtrates produce destruction of the principal islets of certain species of teleost fish It is well to note that the normal values they report are surprisingly low Jephcott (personal communication) found an insulin content of 1 4 units per gram pancreas in a monkey dying from massive tuberculosis, whereas values in normal monkeys averaged 2 5 units per gram Murray and Waters (38) induced infection in dogs by ligating the appendix and wrapping it with omentum They concluded that their results showed "that there is a significant decrease in the insulin content of the pancreas of dogs suffering from an acute infection associated with fever and accompanied by suppurative processes " It might be pointed out that the normal values they give are high and that the concentrations in the infected animals were within the range of normal values reported by other workers It is possible that this reduction may simply reflect a diminished food intake, since undernutrition itself has subsequently been shown to lower insulin content (63) Some insulin assays done in our laboratory for T F Nicholson showed that the insulin content of pancreas in rats injected with staphylococcus toxin was not significantly different from that of control animals receiving the same caloric intake

*Insulin and tumors* Cori (64) was unable to find more than a trace of insulin-like substance in a variety of benign and malignant tumors Cramer, Dickens and Dodds (65) found also that a number of different tumors yielded no appreciable amount of insulin

Ruffo and Correa (66) isolated a substance from spindle-celled sarcomata in rats which when injected lowered the blood sugar level While the existence of insulin in tumor tissue in general seems unlikely, its presence in certain pancreatic tumors and their metastases is well established Wilder, Allen, Power and Robertson (4) reported a case with carcinoma of the islets of Langerhans and metastatic tumors in the liver Tumor nodules taken from the liver were extracted and tested for insulin They found from 1/4 to 1/2 as high an insulin concentration in the metastatic tumor tissue as they normally obtained from pancreas (0 4 clinical unit (0 13 unit) per gram) Power, Cragg and Lindem (67) also reported that insulin was found in metastatic islet cell tumors in the liver Derick and associates (68) found that a saline extract of an islet cell tumor had an insulin-like effect, and Graham and Womack (69) estimated that

4 units of insulin were present in 1 gram of tumor tissue from one of their patients. Campbell, Graham and Robinson (70), reporting on several cases with islet cell tumors of the pancreas, found that the insulin concentration in the tumors was 4–40 times that of the normal pancreas. A malignant tumor arising from aberrent pancreatic tissue in the liver was shown by Ballinger (71) to contain an insulin like material.

It has already been pointed out that the calculated concentration of insulin in *islet* tissue for the Wistar rat is 119 to 193 units per gram. Using an $\frac{\text{islet} \times 100}{\text{acinar}}$ ratio of 1.62 for humans at 21 years of age, calculated from Ogilvie's data (54) and insulin concentrations in normal human pancreas obtained by Scott and Fisher (53), the insulin concentration in pure human *islet* tissue is found to be 107 units per gram on the average and as high as 238 units per gram in individual cases. These are only very approximate values but they serve to show that the insulin levels for islet tumor tissue published in most reports are lower than would be expected. However, recently D. A. Scott, assaying the insulin content of an islet cell tumor provided by W. R. Campbell and R. R. Graham, found a value of 214 units per gram of islet tumor tissue (total weight of tumor 3.3 grams). This concentration is not greatly different from the calculated value for the *islet* tissue of normal human and rat pancreas. The concentration is approximately that which could be expected if the tumor were pure islet tissue.

*Zinc and insulin content.* Because of the association of zinc with insulin crystals and with commercial preparations of insulin, and because of fibrotic changes in the pancreas resulting from zinc feeding (72), Scott and Fisher (16) undertook to determine if there were any significant relation between the insulin and zinc content of the pancreases of rats maintained on basal diets and diets containing added zinc. They found that the ingestion of large amounts of zinc caused an increase in the total zinc content of the pancreas but no significant change in the total insulin content of the pancreas (basal 11.52 units, zinc diet 10.40 units). It is interesting to note, however, that the average *concentration* of insulin in the pancreas was considerably higher in the group given zinc (basal 1.08 units per gram, zinc diet 2.56 units per gram), but the weight of the pancreas was only about half that of the control group. This illustrates the importance of the way in which results are expressed. If these workers had presented their results only in terms of concentration of insulin per gram of pancreas, an erroneous conclusion concerning changes in insulin content might have been reached because of the great reduction in the acinar tissue.

DIET AND THE INSULIN CONTENT OF THE PANCREAS. Some of the most interesting changes in the insulin content of the pancreas are those related to alterations in the diet. Most of these concern the effect of diminishing the caloric intake or reducing the amount of carbohydrate ingested. Certain other dietary factors, however, have been investigated.

*Fasting and undernutrition.* One very definite change in the insulin content of the pancreas is that produced by fasting. Best, Haist and Ridout observed that in rats starved for seven days the insulin content of the pancreas was much

lower than in control animals with the same initial weight (23) (73) The weight loss in the fasted rats was 23 per cent of the initial body weight, the insulin content for these animals was 14 1 units per group of 10 rats and that for the controls, 26 5 units per group Reducing the intake of a balanced diet in male rats was found by Haist and Best (63) to bring about a reduction in the insulin content of the pancreas With a daily intake of 15 calories per 225 gram rat the results were practically the same as for complete starvation With an intake of 45 calories the value was nearer the control level though, on the average, less than for the group receiving 65 calories per 225 gram rat Undernutrition itself thus may influence the insulin content of the pancreas, though these data do not rule out the possibility that even when using different amounts of a balanced diet the available carbohydrate may be the limiting variable

In dogs the effect of fasting is more difficult to demonstrate than in rats The animals do not form as homogenous a group as the rats, and the equivalent fasting period is much longer Haist, Campbell and Best (74) report that in dogs fasted for nine days the insulin values were in the lower part of the normal range Aubertin, Lacoste, Saric and Castagnou (75) claim that fasting increased the islet volume in dogs and that in two dogs fasted for 15 and 43 days the insulin concentration in the pancreas was elevated Since the normal values they give, however, (0 15 to 0 23 unit per gram of pancreas) are about one-tenth of those commonly reported and since the normal variation in dogs is great, their results may be questioned According to Foglia (76) the pancreases from dogs fasted 2 to 3 weeks when grafted into the necks of depancreatized dogs showed no impairment of the ability to reduce blood sugar in four cases, a slightly diminished ability in one and a very reduced ability in two others These findings in general correspond with the results of insulin assay in dogs and might be a reflection of the insulin concentration in beta cells (See the discussion in the section on the pituitary gland )

*Fat, sugar and protein* Feeding a diet very rich in fat (90 per cent fat + agar, salt mixture and vitamins) gives a reduction in the insulin content of the pancreas comparable to that obtained with fasting (23) (73) Some of our unpublished data indicates that this effect of fat-feeding on insulin content is still obtained if fat in the form of olive oil is given by stomach tube in amounts equivalent to the normal total caloric intake The effect is obtained also even though the accumulation of liver fat is prevented by the daily administration of 100 mgm of choline chloride per rat Best, Haist and Ridout (23) previously reported that in rats poisoned with carbon tetrachloride the insulin content of pancreas was not related to the level of liver fat The effect of fat-feeding on insulin content therefore cannot be ascribed merely to a poor caloric intake or to an altered liver function associated with the accumulation of fat

A diet moderately rich in fat (fat 46 per cent, protein 15 per cent, carbohydrate 28 per cent by weight) appears to give a slight decrease in the insulin content of the rat pancreas, but the findings do not suggest that an extensive depletion of insulin would be produced readily by a diet of this type (23) The demonstration of the effect of fat-feeding in dogs is accompanied by the same difficulties

as the demonstration of the effect of fasting Haist, Campbell and Best (74) report values in the lower part of the normal range for 3 dogs fed fat for eight days

Since fasting and fat feeding give similar reductions in insulin content the possibility exists that the lowering of content in both instances results from a decreased supply of carbohydrate Best, Haist and Ridout (23) showed that when equicaloric rations of sugar and fat were given, the insulin content of the pancreas remained higher in the sugar fed animals than in those fed fat The difference amounted to 42 per cent of the higher value despite the fact that the weight loss was comparable in the two groups There was a noticeable reduction in insulin content in the sugar fed group, due probably to a reduced caloric intake Feeding a diet moderately high in carbohydrate for a period of 3 to 7 months gave no appreciable change in insulin content (23) Other observers confirm this finding that high carbohydrate diets do not greatly affect insulin content (77) A diet composed almost entirely of protein appears to have an effect on insulin content intermediate between that of carbohydrate and that of fat (23) Some of our unpublished data indicate that rats receiving a diet in which gelatine is the protein have a lower content of insulin in the pancreas than those receiving equicaloric rations of the same diet with casein replacing the gelatine The diet was otherwise balanced Four 'casein" groups gave an average content of 21 6 units per group of 10 rats, whereas four 'gelatine' groups receiving the same caloric intake had an average content of 15.2 units per group of 10 rats In all instances the animals receiving the gelatine diet lost weight while those on the casein diet gained weight It is possible that any diet not adequate for growth or maintenance would give similar results and that it is not a specific effect for a particular amino acid deficiency

Carbohydrate tends not only to maintain the level of insulin content but also to restore the level following a period of starvation When rats are starved for 7 days and then given a balanced diet, the insulin content of the pancreas returns to normal within 7 days When they are given a diet of sugar the content is brought back within the normal range but the level is not maintained Fat leads to no elevation in content above the level in the fasting animal but, if anything, causes a further reduction (23) (73)

Casein has an effect similar to sucrose when fed to rats previously starved for 7 days At the end of 7 days of re-feeding the value for the sugar fed rats averaged 10.8 units per group of 10 rats, and for the casein fed 18.3 units per group In two experiments in which dextrose was given in amounts equivalent to 50 per cent of the casein intake, the casein groups averaged 20.3 units per group of 10 rats, and those receiving dextrose 17 7 units per group (unpublished data)

The data already presented would justify the conclusion that the effect of diet on the insulin content of the pancreas is due in large part to the amount of available carbohydrate in the diet, although certain other factors are not excluded

*Insulin, fasting and fat feeding* In an attempt to throw some light on the

way in which the effect of fasting or fat-feeding was brought about, insulin was injected into fasting and fat-fed rats (13) (78) The insulin administration enhanced the effect of fasting and fat-feeding, causing a reduction in the insulin content of pancreas to still lower levels Best and Haist conclude "it is improbable that the insulin liberation by islet cells would be increased when insulin is given The insulin production by the islet cells is presumably reduced Since insulin administration enhances the effect of fasting or fat-feeding, the effects of insulin administration, fasting and fat-feeding are similar These procedures, i e , insulin administration, fasting and fat-feeding, apparently rest the pancreatic islet cells by reducing the need for endogenous insulin " The insulin-treated, fasted and fat-fed rats which have a low insulin content of the pancreas, show a *reduction* in the specific granules of the beta cells of the islets of Langerhans (11) but no evidence of hydropic degeneration This further emphasizes the close relation between the specific granulation of beta cells and insulin content The influence of fasting and fat-feeding in preventing the effects of diabetogenic anterior pituitary extracts on pancreatic insulin content (74) and in alleviating the effects of extensive partial pancreatectomy (6) provides further evidence that these procedures "rest" the islet cells Whether or not in normal animals fasting, fat-feeding and insulin administration leave the islet cells with a greater functional reserve, a greater potentiality for increased function without damage, cannot be decided at present

*Endocrines, fat-feeding and fasting* Himsworth and Scott (83) contend that fat-feeding and fasting influence sugar tolerance and insulin sensitivity by increasing the activity of the pituitary gland Chambers, Sweet and Chandler (84) found, however, that starved hypophysectomized dogs showed a definite hyperglycemia and an absence of rise in respiratory quotient following glucose administration That is, they responded in a manner similar to normal animals and hence the pituitary probably is not involved in the phenomenon The pituitary also does not seem to be concerned with the effect of dietary changes on the insulin content of pancreas The insulin-lowering effect of fat-feeding can still be obtained after the removal of the pituitary gland (13) (85) and, in the hypophysectomized animal, after the insulin content of pancreas has been reduced by the feeding of fat it can be restored by giving a balanced diet (13) (85) The reduction in insulin content by fat-feeding can be obtained also in adrenalectomized and gonadectomized rats (25) (86) These findings indicate that the pituitary, adrenal glands and gonads are not fundamentally involved in the influence of fat-feeding on the insulin content of the pancreas The pancreas appears to be able to regulate production and liberation according to the need for insulin in the absence of these glands

*Relation to other observations on diet and carbohydrate metabolism* When an animal is starved or when certain diets are given, definite changes in the response of the body to administered carbohydrate become evident There is a progressive decrease in sugar tolerance, especially marked in the intermediate period and improving again in the final stage of fasting This decreased tolerance is accompanied by excretion of sugar in the urine and an absence of the normal rise

in respiratory quotient after glucose administration For the relevant literature concerning these changes the reader is referred to a comprehensive review by W H Chambers on Undernutrition and Carbohydrate Metabolism (79) Haist, Campbell and Best (74) discuss the relation of the changes in insulin content to these observations They suggest two possibilities *1*, that when the islet activity is reduced, the immediate response of islet cells to any sudden increase in the need for insulin is less than normal, or *2*, when the level of insulin liberation is low, that is, when the continuous outpouring of the hormone is reduced, the tissues and especially the liver are altered so far as their response to sugar is concerned A third possibility is that even though the low insulin content is associated with conditions causing the above phenomena, islet function is not involved in the phenomena Soskin (12) cites evidence to indicate that "in the presence of a sufficiency of insulin, but not necessarily an extra secretion from the pancreas, the normal liver, as one of its responses to administered dextrose, decreases the output of blood sugar which it has been previously supplying from its own resources" Soskin, Essex, Herrick and Mann (80) were able to show by direct measurements that the liver played an important rôle in the regulation of blood sugar level by taking up sugar from the blood or liberating sugar into the blood as needed Hence the response of the liver to the blood sugar level would seem to be a very important factor in sugar tolerance For a discussion of this subject see the review by Soskin (12) Soskin considers that insulin helps to determine the threshold of the homeostatic mechanism of the liver, i e , the level at which the hepatic inhibitory response is obtained He does not show however, that with a lower than normal continuous insulin supply no change in sugar tolerance results In human diabetics and depancreatized dogs, Ricketts (81) obtained diabetic tolerance curves in response to breakfast despite the fact that a dose of protamine insulin given on the previous evening was sufficient to keep the blood sugar normal through the following morning if no food were taken Others report results at variance with these (12) (82) Soskin suggests that an extra secretion of insulin ordinarily resulting from hyperglycemia, while not essential, probably acts as a factor of safety in increasing the efficiency of blood sugar regulation (12) While the rôle of the pancreas in the control of the blood sugar level is controversial, it seems fair to conclude at least that physiological changes in islet function may lead to quantitative changes in the blood sugar regulation

*The effect of vitamin deficiency* Very little work concerning the effect of vitamin deficiency or excess on the insulin content of pancreas has been reported Best Haist and Ridout showed that rats receiving a diet complete except for certain vitamins, and containing vitamins A and D in the form of cod-liver oil concentrate had a reduced insulin content of pancreas, probably due to the diminished caloric intake A second group fed the same caloric intake but having vitamin $B_1$ added to the diet had an insulin content of pancreas not significantly different from the first The addition of $B_1$ thus seemed to be without effect on insulin content (23) Further systematic studies in this field should be undertaken

ENDOCRINES AND INSULIN CONTENT While there is at present no evidence that the endocrine glands exercise any *fundamental* normal control over the insulin content of the pancreas, yet in certain instances preparations obtained from them do greatly alter the insulin content For that reason it is important to know which glands affect the insulin content of pancreas and under what circumstances the influence is noted

*Adrenals* The adrenal glands can be removed without greatly altering the insulin content of pancreas Fraenkel-Conrat, Herring, Simpson and Evans (77) in one experiment reported that the pancreases of adrenalectomized rats contained more insulin than unoperated rats, but Haist and Bell (25) (86) found that adrenalectomized rats maintained on sodium chloride showed no significant change in the insulin content of pancreas as compared to control animals receiving the same caloric intake (paired-fed controls, 12 4 units per group of 10 rats, adrenalectomized, 12 5 units per group of 10 rats) It has been reported also that the administration of adrenal cortical extract gives little change in the insulin content of pancreas (86) (77) The finding that the diabetic state of partially depancreatized rats can be increased by certain adrenal steroids (87) (88) and the report that glycosuria, though not true diabetes, can be produced in normal rats by the administration of 17-hydroxy-11-dehydrocorticosterone (89) make it seem possible that if certain specific adrenal steroids were used in sufficient quantities, some measurable effect on insulin content might be evident Fraenkel-Conrat et al (77) observed that adrenocorticotrophic pituitary preparations increased the insulin content of rat pancreas If this were due to the adrenocorticotrophic effect then an adrenal influence on insulin content would be indicated They believe, however, that the effect was the result of contamination with lactogenic hormone The fact that the insulin-reducing effect of fat-feeding and the restoration of insulin content by a balanced diet can be obtained after adrenalectomy is good argument for a normal regulation of pancreatic insulin that is independent of the adrenal glands (25) (86)

*Gonads* Sex differences in the insulin content of pancreas are not striking Fraenkel-Conrat et al (77) reported a higher average insulin content in normal female than in male rats, though there was great overlapping in the range of values This difference was not observed after hypophysectomy The data of Haist and Bell (25) indicate that, considered over a wide range of body weight, males and females have roughly the same insulin content of pancreas There are differences, however, in the effect of certain procedures on the insulin content in the two sexes For example, horse pituitary extract is pancreatrophic in the female but not in the male rat unless the gonads are removed (90) The insulin content of the pancreas is not significantly altered by removal of the gonads and the insulin-reducing effect of fat-feeding can still be obtained in their absence (25) However, certain materials obtained from the gonads or simulating their effects can cause a great change in the insulin content of pancreas

Griffiths and Young (91) report that implantation of tablets of oestrogen in rats interferes with normal pituitary function and depresses growth The pancreas is not reduced in proportion to the body as a whole and the pancreatic

insulin is significantly increased in relation to the body weight An elevation in the insulin content of the pancreas in rats is reported to result from the administration of the oestrogens, oestrone (92), oestriol (93) oestradiol (93) (94) oestradiol dipropionate (95) and stilboestrol (93) (94) The insulin content of the rabbit pancreas is increased by hexoestrol (34) (96) Progesterone has no insulin-increasing effect (94) and the androgen testosterone does not induce an elevation in the insulin content of pancreas in rats (93) In fact, some claim that the content is decreased by testosterone (94) Griffiths reported that alpha methyl stilbene closely related to hexoestrol and stilboestrol, greatly increased the pancreatic insulin in the rabbit but was non-oestrogenic (34) (96) The increase with this material was tremendous (control, 5.2 units per gram, injected 24 5 units per gram) Since the rabbit pancreas is diffusely distributed and since only portions of the pancreas and not the whole gland were used for the determinations, serious error might arise from this source Because the alpha methyl stilbene was not oestrogenic Griffiths concluded that the insulin increasing properties of hexoestrol and stilboestrol "have little to do with their oestrogenic properties" Fraenkel-Conrat and associates (95) showed that after hypophysectomy the insulin-increasing effect of oestradiol dipropionate was not obtained The insulin content of the pancreas was elevated by the implantation of pituitary glands obtained from rats receiving oestrogens, while pituitaries from control rats did not have this effect These experiments suggest that the pituitary mediates the insulin-increasing activity of oestrogens

Vazquez-Lopez (97) gives as histological evidence of excessive secretory activity of the islet cells after injections of oestrogens the demonstration of marked enlargement of the Golgi apparatus, and harmonizing with the results on insulin content are the reports that injections of oestrin cause an increase in the islet tissue in the pancreas of certain species (98) (99) Castration has been reported to lead to hyperplasia of islet tissue in guinea pigs, though the administration of testosterone apparently had no effect on pancreatic morphology (100) Others contend that, in the dog, injections of testosterone propionate lead to a reduction in islet tissue (101) The histological effects of testosterone seem to be no more definite than the effects on pancreatic insulin Owing to the fact that the islets vary greatly in size and number among members of the same species, erroneous conclusions may be drawn from estimates of the effect of injected materials on the islets unless some good quantitative measure of islet volume is used Even when this is done the volume of the beta cells, which is the important value, still remains undetermined

*Thyroid* Various claims have been made concerning an interrelationship of the thyroid gland and the pancreas Fraenkel-Conrat and associates (102) emphasize the influence of dosage on the effect of thyroxin, for example, with low doses thyroxin synergizes with growth hormone while with high doses weight loss occurs These workers tried the effect of thyroxin in hypophysectomized rats, giving doses sufficient to restore the oxygen consumption to normal. No weight loss occurred, but the insulin content of the pancreas was reduced to about half that of the controls In one experiment performed on normal rats

the administration of thyroxin led to an increase in pancreatic insulin Some observers have reported that the injection of thyroxin and thyroid extract into normal animals gives hyperplasia of the islets (103) but this was not confirmed by others (104)

*Pituitary* A relationship between the anterior pituitary gland and the *pancreatic islets* had been suspected because of the high incidence of diabetes in certain conditions associated with pituitary dysfunction (105) Johns, O'Mulvenny, Potts and Laughton in 1927 (106) reported that hyperglycemia, glycosuria and polyuria had been produced in dogs by injecting extracts of the anterior pituitary gland Injections of anterior pituitary extracts were shown to produce diabetes in intact experimental animals by Evans, Meyer, Simpson and Reichert (107), Baumann and Marine (108) and Houssay, Biasotti and Rietti (109) Evans and associates noted a persistence of the diabetic state after the administration of the extract was discontinued, but the significance of these findings was not generally appreciated until F G Young showed conclusively that if the course of injections of anterior pituitary extracts was sufficiently severe and prolonged, the diabetes persisted indefinitely after the injections were discontinued (110) The pancreases in these "permanently" diabetic animals contained fewer than normal islets (111) (112) (113) (114) (115) (116) Granule staining showed that the lack of granular beta cells was almost complete (8) (9) (111) (115) Campbell and Best (112) reported that the insulin content of the pancreas of the permanently diabetic dog was extremely low (less than 2 units per total pancreas), a finding that was later confirmed (7) (117)

Best, Campbell and Haist (7) observed that during the course of the injections of the anterior pituitary extract, before the diabetic state had become "permanent," there was a progressive reduction in the insulin content of the pancreas When, after 7 daily injections, the administration of the extract was discontinued, the insulin content of the pancreas returned to normal If the course of injections of the extract was sufficiently severe, then cessation of the injections was not followed by restoration of the insulin level in the pancreas, the content remaining low for as long as 198 days Ham and Haist (8) (9), reporting on the histological changes in the pituitary-injected animals, showed that there was a progressive degranulation of the beta cells of the islets of Langerhans that paralleled the fall in insulin content The degranulation was followed by hydropic degeneration of the beta cells With the cessation of the injections of anterior pituitary extract the beta cells regained their normal granulation, the return of granulation corresponding to the restoration in insulin level (9) Richardson and Young (111) had previously reported cellular proliferation and hydropic degeneration of the beta cells of the islets in 2 dogs during the course of anterior pituitary injections

The islet changes in the pituitary-injected dogs closely resemble those reported by Homans (5) and Allen (6) for partially depancreatized animals Also, the fall in insulin concentration in the pancreatic remnant in partially depancreatized animals is similar to the change in insulin level found in the animals receiving injections of anterior pituitary extracts Allen (6) concluded, and Bell, Best

and Haist (14) support the view, that the islet changes in the partially depancreatized dog are due to overwork of the beta cells of the islets with consequent exhaustion. Haist, Campbell and Best (74) discuss the cause of the alterations in the animals with pituitary diabetes and conclude that here too the islet changes result from an exhaustion through overwork. In these animals the overwork probably results from several different influences. The diabetogenic pituitary extracts have profound extra pancreatic effects. They can exhibit their hyperglycemic and glycosuric action in the absence of the pancreas (118) (119) (120) and the action of insulin is antagonized even before the blood sugar is elevated (121) (122) (123). Many different trophic principles may be involved since organs and tissues other than the pancreas show cellular proliferation and evidence of increased activity while the extracts are being given (8) (9). Best, Campbell and Haist (7) report an interesting inverse relationship between the fasting blood sugar level and the concentration of insulin in the pancreas of pituitary-injected dogs. When the injections of the anterior pituitary extract were discontinued, the blood sugar fell rather quickly to normal values but the insulin content of the pancreas remained low until after the normal blood sugar level was restored. These findings seem to suggest that the blood sugar level or some extra pancreatic factor causing the elevation in blood sugar, is the cause of the islet changes rather than the reverse. The experiments are not conclusive in this regard and these authors *do not assume* that the elevated blood sugar level is the main factor stimulating insulin secretion. It seems reasonable to conclude, however, that as a result of the extra pancreatic effects of the extract the need for insulin is increased. The fact that the effects of anterior pituitary extract on insulin content and pancreatic islets can be prevented to some extent by procedures such as the administration of insulin (10) (124) (125), fasting and fat-feeding (74), which presumably reduce the need for endogenous insulin, argues in favor of the view that the effects of pituitary injections on islets are due to overwork. Ham and Haist (9) suggest, however, that in addition to increasing the need for insulin, the anterior pituitary extracts may remove restraints which ordinarily hold secretory and growth activities within certain limits. Hence a direct effect on the islets may operate along with extra-pancreatic influences by permitting or encouraging the excessive overwork which leads to exhaustion. Marks and Young (17) also speculate on the manner in which the pituitary effect is brought about.

Houssay and his associates (126) (127) grafted the pancreases of dogs exhibiting pituitary diabetes into the necks of depancreatized animals. They concluded on the basis of the effect on blood sugar that there was a diminished liberation of insulin from these pancreases. The conclusion that there is a diminished capacity for secretion in these pancreases seems reasonable. However, it is difficult to evaluate the results. Since the pancreatic transplants have a limited life one might wonder whether or not some deterioration of the tissue may begin at the time the transplant is made. Since insulin can be extracted from the pancreas by perfusion (128) it is possible that insulin is being washed out of the pancreatic transplant by the blood of the recipient animal

The amount washed out would depend upon the insulin concentration in the islets and the relation of this concentration to the blood insulin level Such a test, then, might merely reflect the insulin content of the transplanted pancreas However, it does seem likely that once hydropic degeneration occurs or permanent diabetes with islet atrophy has been established, the liberation of insulin by the pancreas is decreased, and it probably is reduced before this time when the content of insulin is low and marked degranulation of the islet cells is evident

It is not within the scope of this paper to discuss fully the nature of the pituitary diabetogenic factor or factors A marked reduction in the insulin content of the pancreas is associated with the diabetic effects in the dog (7) (112) (129) (17), and a loss of diabetogenic activity is accompanied by an inability of the extracts to reduce the insulin content (17) Crude saline, alkaline and acid extracts of fresh anterior pituitary glands (130), picric acid preparations (131), pseudoglobulin (17) (132) and globulin fractions (7) (133), growth (107) (108) (134) (135) and ketogenic (133) (134) preparations have been shown to be diabetogenic in dogs

The diabetogenic factor closely accompanies the growth factor in its preparation, and Shipley and Long (134) suggest that the ketogenic, growth and diabetogenic factors may be identical Young (136) observed also that pituitary diabetogenic preparations usually have growth-promoting activity However, Marks and Young (17) found that "stale" crude extracts of fresh pituitary glands retain growth-promoting activity but are not diabetogenic and they conclude that the diabetogenic and growth-promoting substances are not identical Fraenkel-Conrat and associates (77) found that the administration of growth hormone brought about a decrease in the insulin content of the pancreas in the rat, but they were unable to produce diabetes by its administration This effect of growth hormone was still obtained after adrenalectomy but not after removal of the pituitary The importance of the growth factor in the diabetogenic action is again emphasized in the recent work of Marx, Anderson, Fong and Evans (136a), who report that purified growth hormone preparations practically free from lactogenic, adrenocorticotrophic, thyrotrophic and gonadotrophic factors greatly increase the urinary excretion of glucose in partially depancreatized, sugar-fed rats

Albumin and euglobulin fractions of anterior pituitary are not diabetogenic (132) and the diabetogenic effect apparently is not produced by relatively purified preparations of prolactin (133) (134) (137) (138), thyrotrophic (138), gonadotrophic (136) (139) or glycotropic (136) factors Since Houssay and associates showed that the diabetogenic action can be obtained in some species in the absence of thyroid, gonads and adrenals (140) (141), it is difficult to see how a pituitary principle acting on these glands separately could be responsible for the total diabetogenic activity The adrenal gland, however, does seem to be involved in some way in the diabetogenic effect Long and Lukens (142) (143) found that after removal of the adrenals the diabetogenic effect of pituitary extracts was not obtained in cats The diabetogenic effect has been observed by Houssay and Biasotti in partially depancreatized, adrenalectomized dogs

maintained in good condition with cortical extract (144), desoxycorticosterone or sodium chloride (141) It would seem then that the adrenals are important in the production of the diabetogenic effect, though some effect not mediated by the adrenals must also be granted (141) Of all the organs in the body, the liver is most necessary for the diabetogenic effects of pituitary extracts (107) (141) (145) The occurrence of diabetes as a result of pituitary injections probably depends not on a single pituitary factor but on a variety of effects, many of them "trophic" in nature (9), exerted on a number of different organs and tissues of the body Long (146) considers the possibility that there may be two constituents of the diabetogenic factor, one, heat-stable, able to cause glycosuria in a hypophysectomized-depancreatized animal, and one, heat-labile, required to give ketonuria and glycosuria in the intact animal Young (136) concurs in this view and suggests that the heat-stable factor may be responsible for the extra-pancreatic effects

Certain pituitary preparations causing a reduction in the insulin content of pancreas in the dog occasion an increase in the insulin content of pancreas in the rat (17) (33) (77) (92) (94) (129) Anselmino, Herold and Hoffmann (147) had reported that following the injection of an anterior pituitary extract there was an increase in the size of the islets of Langerhans Some confirmed, but others were unable to repeat their results Richardson and Young (30) showed however that injections of crude extracts of the anterior pituitary gland caused an increase in the volume of the islets in Wistar rats The increase in the insulin content of pancreas that results from the injection of anterior pituitary extracts in rats might be expected then, in view of the increase in islet volume Marks and Young (17) state that evidence is lacking to show that the insulin increasing and islet-increasing factors are the same, but intimate that until evidence for a plurality of the factors is forthcoming, the provisional assumption may be made that one substance is responsible for both effects

Signs of proliferation are evident in the islets of dogs as well as of rats injected with anterior pituitary extracts Richardson and Young (111) were the first to note evidences of cell division in the islets of Langerhans in pituitary injected dogs Ham and Haist (8) (9) found proliferation not only of the islet cells but also of acinar cells and the "mother cells" of the small ducts of the pancreas in dogs receiving pituitary injections Even in the dog, then, there are some proliferative effects of the extracts, though here the degranulation and degeneration seem to predominate and the insulin content of the pancreas falls

The mouse resembles the rat showing an elevation in the insulin content of pancreas when crude extracts of pituitary are used (26) The rabbit exhibits a fall like the dog, though usually not so great (26) (131)

Considerable work has been done in an effort to separate the diabetogenic (dog) and insulin increasing or pancreatrophic factors (rat) Marks and Young (17) showed that a relatively purified diabetogenic, psuedoglobulin fraction of anterior pituitary extract resembled a crude extract in having diabetogenic insulin increasing and growth promoting effects 'Stale" crude extracts of fresh anterior lobe tissue (extracts which had been standing 24 hrs at room tem-

perature or which were incubated for 5 hrs at 37°C) were found to have little or no diabetogenic effect, though they did have growth-promoting and insulin-increasing activity Extracts of commercial acetone-desiccated anterior pituitary glands with no detectable diabetogenic or growth-promoting activity were shown to increase the insulin content of the rat pancreas While Marks and Young felt that the insulin-increasing (in rats), diabetogenic (in dogs) and growth-promoting substances were not identical, they were unable to obtain diabetogenic fractions free from growth-promoting or insulin-increasing activity, or to prepare growth-promoting extracts free from insulin-increasing activity They state that their observations "do not preclude the possibility that the anterior lobe tissue contained a substance (pseuglobulin?) having all three activities (diabetogenic, growth-promoting and insulin-increasing), and that differential inactivation of prosthetic groups concerned in the different activities may take place" Later in the paper, however, they conclude that "there are certainly two principles"

Funk and associates (94) found that a protein fraction, prepared by alkaline extraction of anterior pituitary glands previously extracted with 10 per cent salt solution, increased the insulin content of pancreas Fraenkel-Conrat and associates (77) report that unfractionated alkaline anterior pituitary extracts only increased the insulin content of pancreas in excessive doses Globulin fractions of these extracts had no effect Their report concerning a decrease in the insulin content of pancreas in the rat when growth hormone was administered has been mentioned previously This observation supports the contention of Marks and Young (17) that the growth-promoting and insulin-increasing factors are not identical

Marks and Young (92) conclude that the insulin-increasing factor is not identical with the gonadotrophic principle First, ox prolactin preparations which contained only a trace of gonadotrophic principle had insulin-increasing effects They found, moreover, (90) that horse pituitary extracts which were actively gonadotrophic did not elevate the insulin content of pancreas in male rats, though they were active in females Ox pituitary extracts, which were only moderately gonadotrophic, gave an elevation in the insulin content in both male and female rats In this connection they showed that treatment with testosterone inhibited the insulin-increasing action of ox pituitary extract in male rats Since they found that extracts of horse pituitary actively increased the insulin content of the pancreas in the female but not in the male animal they considered that the effect of the endogenous testosterone might explain the failure of horse pituitary (actively gonadotrophic) to influence the insulin content of pancreas in male rats Moreover, both the ox and the horse anterior pituitary extracts had an insulin-increasing effect in gonadectomized male and female rats This, plus the fact that testosterone itself did not induce a rise in insulin content, led them to conclude that the gonads do not mediate the pituitary effect (92) The work of Fraenkel-Conrat et al (95) would indicate that, on the contrary, the pituitary probably mediates the insulin-increasing activity of certain gonadal substances (oestrogens)

Marks and Young (92) found that prolactin was very effective in increasing the insulin content of the pancreas in the rat but considered that it was improbable that prolactin and the pancreatrophic principle were identical. They thought this for several reasons. First, pseudoglobulin fractions of ox pituitary had insulin-increasing effects and yet they were free from detectable prolactin. Secondly, the daily dose of pituitary extract required to give an insulin-increasing effect contained only ⅓ of the effective daily dose of prolactin. Finally, sheep pituitary extracts were only half as potent as ox pituitary extracts in giving the increase in insulin content, and yet the sheep pituitaries were richer in prolactin. Fraenkel-Conrat and associates (77) found that lactogenic preparations which were chemically pure, as judged by electrophoretic, ultracentrifuge and solubility studies, increased the insulin content of pancreas. The lactogenic preparations were effective in 3 out of 5 tests on normal animals when 1 mgm or more daily was given for at least 17 days, and were similarly active in hypophysectomized rats. Adrenocorticotrophic preparations which were also effective in increasing insulin content were contaminated with lactogenic hormone up to 25 per cent and these workers felt that the effect was probably due to the contaminating lactogenic hormone. Funk and associates (94) report that highly purified prolactin reduces the insulin content of rat pancreas, but Fraenkel-Conrat et al. (102) were unable to confirm this effect, finding only an increase when pure lactogenic hormone was used under the conditions reported by these workers.

Marks and Young (92) conclude that the thyrotrophic factor is not responsible for the increase in insulin content, since only traces of it are present in the prolactin fraction which is effective in that regard. Fraenkel-Conrat and associates (77) showed that a purified thyrotrophic preparation was not active in elevating pancreatic insulin. The fact that thyroxin itself lowers the insulin content of pancreas in hypophysectomized rats (102) may also be taken as evidence against the view that the thyrotrophic principle is the responsible factor. The material causing the increase in insulin content is relatively unstable to heat and, according to Marks and Young (92), loses most of its activity when heated to 100°C for a short time. This serves to differentiate it from the glycotropic factor which is relatively heat-stable. They found also that alcohol-dried pituitary and an alcohol-soluble fraction of ox pituitary were not significantly pancreatrophic when injected or given by mouth.

The results of experiments on hypophysectomized animals assist in the evaluation of the rôle of the pituitary in the normal control of islet activity. Chambers, Sweet and Chandler (84) reported that the insulin content of the pancreas in hypophysectomized dogs did not differ from that of normal animals. Haist and Best (13) (85) showed that while the insulin content of the pancreas in hypophysectomized rats was slightly less than that of control animals fed ad libitum, it did not differ significantly from that of control animals receiving the same caloric intake. Fraenkel Conrat and associates (77) also observed that there was no significant difference between the insulin content of normal and hypophysectomized female rats. In 80 gram hypophysectomized rats, Griffiths

and Young (24) (91) report an increase in the insulin content of pancreas, but in hypophysectomized rats of 100 grams or more the amount of insulin per 100 grams of body weight remains virtually the same as for normal controls According to Krischesky (148) the ratio of islet tissue to body weight in the *male* rat is increased by hypophysectomy Haist and Best (13) (85) found that in some respects at least hypophysectomized rats respond like control animals, since they exhibit a similar reduction in the insulin content of pancreas when fat is fed In these animals the low values following fat-feeding can be brought back to normal by feeding a balanced diet It appears that the pancreas can regulate the production and liberation of insulin according to the need for it, in the absence of the pituitary gland (85) This suggests that the pituitary exercises no *fundamental* normal control over pancreatic islets, despite the effects of injected extracts of that gland

Removal of the hypophysis alters the effect of injections of pituitary materials Crude saline extracts of the anterior pituitary gland have a less striking insulin-increasing effect in hypophysectomized than in normal rats (33) Griffiths and Young (24) (91) indicate that injection of fresh and "stale" anterior pituitary extracts in hypophysectomized rats will induce growth and lead to an increase in the insulin content of pancreas which is approximately proportional to the increase in body weight Griffiths (24) concludes that "although the pituitary gland controls the increase in pancreatic insulin during growth, it may not do so by means of a specific insulin-increasing hormone but rather the increase is associated with the general increase in constituents involved in somatic growth " Fraenkel-Conrat and associates (77) suggest, however, that the existence of opposing balanced influences (insulin-increasing and insulin-decreasing) of the pituitary may afford an explanation of why neither removal nor implantations of the gland produce effects on the pancreatic insulin

From the reports already referred to it will be evident that many anterior pituitary preparations increase the insulin content of pancreas in the rat Crude saline extracts, crude "fresh" alkaline extracts, "stale" alkaline extracts, alkaline extracts of commercial acetone-desiccated glands, pseudoglobulin fractions, alkaline extracts of glands previously extracted with sodium chloride, adrenocorticotrophic and lactogenic preparations are all reported to have this effect In addition to this, the insulin content of pancreas can be elevated in intact rats by the injection of thyroxin or by the administration of the oestrogens, oestrone, oestriol, oestradiol, stilboestrol and oestradiol dipropionate Some of these preparations have been shown to have their significant effect only in the presence of an intact pituitary gland Injections of crude saline and alkaline extracts of anterior pituitary and of the oestrogen, oestradiol dipropionate, do not lead to the usual well-marked elevation in insulin content of pancreas in the absence of the pituitary The lactogenic and adrenocorticotrophic pituitary preparations, however, apparently are effective in hypophysectomized rats The fact that, in the absence of the pituitary, certain preparations are not insulin-increasing, whereas in the presence of the pituitary they do have this effect, would indicate that the insulin-increasing action may be mediated or potentiated by the anterior pituitary gland

If there is a factor having a direct insulin increasing action, it must be demonstrated that the effect of this preparation on the islets is not mediated by the pituitary, thyroid, adrenals or gonads. It must also be shown that the pancreatic changes are not the indirect result of extra pancreatic effects of the preparation. The purified lactogenic and adrenocorticotrophic materials which effectively increase insulin content in the absence of the pituitary come closest to meeting these requirements, but as yet no preparations satisfy all of them.

As far as the insulin content is concerned the effects of the different pituitary preparations already reported are *1*, a great reduction in the insulin content of pancreas in the dog and rabbit, *2*, an increase in the insulin content of pancreas in the rat and mouse. *3*, a decrease in the insulin content of pancreas in the rat. The reduction in the insulin content of pancreas in the dog is associated with diabetes and the extracts producing these effects are said to be diabetogenic. A reduction in the insulin content of pancreas without accompanying diabetes does not constitute a diabetogenic effect. The influence on growth and secretion in the pancreas is called the pancreatrophic effect. The same extract causing diabetes and a reduction of pancreatic insulin content in the dog may cause islet cell proliferation and an increase in the insulin content of pancreas in the rat.

Marks and Young (17) discuss the possible reasons for the difference in the response of the rat and the dog. They state that in the extracts "there are certainly two principles (diabetogenic and insulin increasing), and the observed effects can be provisionally explained on the assumption that the dog is more sensitive than the rat to one, and the rat more sensitive than to the dog to the other." The extra-pancreatic mechanism may be more sensitive in the dog and the influence of the insulin increasing factor may be exerted more readily in the rat. Ham and Haist (9) point out that secretion as well as growth is stimulated by many if not all of the so-called pituitary "trophic" principles. They argue that a pancreatrophic factor, if it acted like other "trophic" materials, would (directly or indirectly) stimulate secretion as well as growth. The same principle then may be involved in the effects on the islets in dogs and in rats. However if the finding is confirmed that globulin fractions and growth preparations (77), which are diabetogenic in the dog, do not elevate the insulin content of pancreas in the rat, then this would consititute evidence in favor of separate diabetogenic and insulin increasing factors.

It seems reasonable to conclude that while many extracts or fractions which are diabetogenic in the dog are insulin-increasing in the rat, the reverse is not true. Many of the insulin increasing preparations are not diabetogenic. This may be due to the necessity of a much more potent solution for the diabetogenic effect than for the insulin increasing action. It is possible that the diabetogenic action results from a combination of many extra pancreatic influences, which increase the need for insulin and indirectly stimulate the islets, along with a direct effect (pancreatrophic ?) which permits or encourages excessive secretion to occur (9). If the pancreatrophic factor is involved one might postulate that new islet cells would be formed. If, at the same time, the extra pancreatic actions of the extract increase the need for insulin greatly in relation to the functional capacity of the beta cells then degeneration would occur in the islets

and this might overshadow the proliferative effects If, however, the extra-pancreatic actions were slight in relation to the functional capacity of the beta cells, then the new formation of beta cells would result in an increase in islet volume and insulin content It has been suggested (9) (74) that if the extra-pancreatic effects of pituitary extracts could be reduced while the proliferative effects were still obtained, then an increase in islet tissue and more nearly normal islet function might result As we shall see in the discussion of the effects of insulin, the administration of insulin along with pituitary extracts tends to prevent the degenerative changes in the islets while allowing proliferation to occur (10) (74) Extracts of anterior pituitary glands which are pancreatrophic but not diabetogenic usually do not improve the diabetes in the *permanently* diabetic dog (135a) However, these animals may be incapable of forming new beta cells (115) (9) It would be important to see if animals found to be responsive to the diabetogenic pituitary extracts could be made resistant to these extracts by pretreatment with pancreatrophic pituitary preparations or by the previous simultaneous administration of anterior pituitary extracts and insulin

*The administration of insulin* Experiments involving the administration of insulin help to throw some light on the manner in which fasting, fat-feeding and injections of pituitary extracts bring about their effects on the insulin content of pancreas Some of the effects concerning insulin administration have already been mentioned briefly in the discussions of the effects of fasting, fat-feeding and of anterior pituitary injections Haist and Best (13) (78) observed that when adequate amounts of protamine zinc insulin are injected into rats receiving the same caloric intake as the controls, the insulin content of the pancreas is reduced The reduction is less pronounced if the insulin-injected rats eat ad libitum Various reports indicate that, following extensive insulin administration, changes in carbohydrate tolerance are evident which are similar to those occurring when fat is being fed or the individual is fasting (149) (150) (151) (152) (153) (154) Lacoste, Aubertin and Saric (155) report that injections of insulin twice daily into dogs lead to an increase in the insulin content of the pancreas The values per kilogram of pancreas which they give are extremely low and their differences do not seem to be significant These observers also noted an increase in the number and volume of the islets in insulin-treated dogs Several other observers report hyperplastic changes following repeated doses of insulin in the guinea pig, dog and white rat (156) (157) (158) (159) (160) Schereschewsky and Moguilnitzky (161) found only congestion of the islets after massive doses of insulin McJunkin and Roberts (162) report that excessive insulin administration in young rats inhibits the proliferative activity of the islet cells although the weight gain of the animals continues to be as great or greater than normal In the pigeon, large doses of insulin induce atrophy of the beta cells of the islets (163) and, in the rat, depressive changes occur (164) With large doses in the rat, Latta and Harvey (165) found a succession of changes leading to shrinkage of the beta cells, followed by practically complete disappearance of the specific granules of these cells and nuclear changes After cessation of the injections the beta cells regained their normal granulation and appearance A

reduction in the specific granules of the beta cells after insulin administration had been commented on previously by Haist and Best (11) Several observers (165) (166) (167) failed to find any evidence of hyperplasia following insulin injections Mirsky and associates (167a) observed that in a few young, partially depancreatized dogs a persistent diabetic condition could be produced by excessive and prolonged insulin administration Foglia (168) reports that the pancreases from dogs receiving repeated injections of insulin plus sugar for 37 days, when transplanted into the necks of depancreatized dogs, brought down the blood sugar as quickly as pancreases from normal animals It should be pointed out that the depressive changes in the islets and in the insulin content of pancreas followed massive doses of insulin, whereas the evidences of hyper plasia usually were reported when smaller doses were used

The changes in the insulin content of pancreas and in the islets when large doses of insulin are injected, appear to indicate that insulin in excess reduces islet function Latta and Harvey (165) state that, following repeated injections of insulin the histological changes indicate an almost complete suppression of metabolic activity in beta cells It seems reasonable to conclude that insulin administration tends to diminish the production and the liberation of insulin by the pancreas If this is true then the fall in insulin content results from a greater decrease in production than in liberation With the resultant lower insulin level in the pancreas, however, liberation is reduced also until production and liberation are again balanced at the lower level Whether the effects of insulin administration are exerted by its influence on blood sugar level or blood insulin level, or indirectly through the pituitary or liver, is not known Since the effect of fat-feeding on the insulin content of pancreas is similar and may still be obtained in the absence of the pituitary gland, the pituitary mediation of the effect is not likely

As has already been pointed out, the injection of protamine zinc insulin into starving rats or into rats fed fat, reduces the insulin content of pancreas to a much greater extent than would result from fasting or fat feeding alone (13) (78) On the other hand the administration of insulin relieves the diabetes in partially depancreatized diabetic dogs and cats and restores the islet cells (169) (170) It prevents the great reduction in insulin content and the degranulation and degeneration of beta cells that would otherwise result from the injection of diabetogenic anterior pituitary extracts (10) (124) (125) and in permanently diabetic cats, restores the islets (125) In pituitary-injected dogs receiving insulin the degenerative islet changes were in most instances prevented, but the proliferative ones were still observed (10) (74) It would seem that the pancreatrophic effect of the pituitary extracts was still obtained in these animals, but that the degeneration of beta islet cells did not occur, presumably because the extra pancreatic effects of the pituitary administration were greatly reduced by the injection of insulin

Recapitulating then, injections of insulin in adequate amounts have been shown to reduce the insulin content of pancreas they enhance the insulin lowering effects of fat-feeding and of fasting in the rat but tend to reduce the effect of

anterior pituitary extract and of partial pancreatectomy on the insulin content of pancreas and histology of the islets in the dog It seems clear that the effects of fasting, fat-feeding and insulin administration are similar in some respects and opposed to the effect of injections of anterior pituitary extract and of partial pancreatectomy The evidence supports the belief that the pituitary extracts and partial pancreatectomy lead to an exhaustion of the islet cells through overwork, whereas the administration of insulin, fasting and the feeding of fat, reduce the need for endogenous insulin and decrease the work of the beta cells of the islets of Langerhans

OTHER TYPES OF EXPERIMENTAL DIABETES The changes in the insulin content of pancreas in pituitary diabetes have been referred to previously Three other methods of producing experimental diabetes should be mentioned These are partial pancreatectomy, the administration of phlorizin and the administration of alloxan

*Partial pancreatectomy* Homans (5) and Allen (6) showed that removal of a large part of the pancreas in dogs led to diabetes associated with degranulation and hydropic degeneration of the beta cells of the islets of Langerhans Concerning insulin changes in partially depancreatized animals, Haist and Best found that when a portion of the pancreas was removed from dogs and the animals did not become diabetic, the concentration of insulin in the remnant remained within the normal range, but when sufficient pancreas was removed so that the animals did become diabetic, then the insulin concentration in the pancreatic remnant fell to very low values (13) (14) Allen (6) attributed the islet changes to exhaustion through overwork, a view supported by the insulin studies (14) Proliferative changes in the pancreas rather similar to those resulting from injections of anterior pituitary extracts have been reported to occur in the pancreatic remnants following partial pancreatectomy in the guinea pig (171) (172), dog (172) (173), and rat (174) Whether or not the changes in pancreatic islets in the partially depancreatized dog are due to a pituitary influence cannot be decided at present In the absence of the pituitary the marked lowering of insulin content in the partially depancreatized dog does not occur, even though the pancreatic remnant is very small (14) Either the pituitary is in some way directly responsible for the effect on the islets or else, in the absence of the pituitary, heavy demands on the pancreas for insulin are not made

*Phlorizin* The administration of phlorizin leads to a state which presents some of the features of diabetes but differs in important respects Phlorizin administration reduces the reabsorption of sugar in the kidney tubules (175) and hence leads to a greatly increased excretion of sugar in the urine The blood sugar level is lowered and there is an increased new formation of sugar in the liver (176)

Nash and Benedict (177) found that extracts of pancreas from phlorizinized dogs lowered blood sugar in a normal fashion and Cori (178) reported no appreciable difference between the insulin content of two phlorizinized and two normal cats These were not particularly accurate assays but indicated that insulin was still present in the pancreases of phlorizinized animals Houssay and Foglia

(179) found that pancreases from phlorizinized animals when grafted into the necks of depancreatized dogs had a diminished ability to restore the blood sugar to normal. They concluded that the insulin secretion was diminished. Lesions in the beta cells of the islets in phlorizinized animals were found by Porto (180). On the basis of these results one might predict that the insulin content of pancreas in the phlorizinized animals would be reduced. Opposed to this is the report that islet hypertrophy has been noted in the guinea pig after phlorizin administration (181).

*Alloxan.* Alloxan, first observed to have a hyperglycemic action by Jacobs (182) has been shown by Shaw Dunn and his colleagues to cause necrosis of islet tissue in the pancreases of rabbits (183) (184). With the development of islet lesions as a result of repeated injections of alloxan, rats develop persistent glycosuria, hyperglycemia and other changes characteristic of diabetes (185). Hughes, Ware and Young (186) showed that in the rabbit the hypoglycemic effect of alloxan can be simulated by the injection of the amount of insulin calculated to be present in the normal rabbit pancreas. They suggest that preformed insulin is liberated by necrotic islet cells to give the hypoglycemia. The islet necrosis in animals injected with alloxan would lead one to expect a reduced insulin content of pancreas. Recently, Goldner and Gomori (187) reported that values for the insulin content of the pancreas in 2 alloxan diabetic dogs were very low, and some preliminary results obtained by Ridout and Wrenshall (personal communication) indicate that the insulin content of pancreas in rats is reduced after alloxan administration. In 48 hours the insulin content had fallen to 0.1 unit per rat in the alloxan treated group as compared to 1.1 units of insulin per rat in the *paired fed* control group.

*Human diabetes and the insulin content of the pancreas.* Pollak (52) found that the concentration of insulin in the pancreas of diabetic humans was much less than normal. His normal values, however, were much lower than those reported later. Scott and Fisher (53) found that the insulin content of the pancreas in 14 nondiabetic human patients averaged 1.7 units per gram of pancreas (173 units per pancreas). In 18 diabetics the insulin concentration averaged less than 0.4 unit per gram of pancreas or a total of less than 40 units per pancreas. One moderately severe diabetic patient kept under control with insulin for many years showed a content of 1.0 units per gram or 162 units total insulin in the pancreas, in other words a normal insulin content. One patient admitted in diabetic coma and not responding to insulin in the 36 hour interval before death, had an insulin content of less than 0.06 unit per gram, or a total of 3 units in the whole pancreas. The high value in the insulin controlled diabetic is in keeping with the results of adequate insulin administration in animals receiving diabetogenic anterior pituitary extracts. The findings in islet studies on the pancreases of human diabetics vary greatly but the general conclusion is that there is no characteristic islet lesion in diabetes. However, as several observers have pointed out while the islets may be normal in appearance in hematoxylin and eosin sections, specific granule stains might show extensive degranulation of beta cells to be present. The insulin concentration closely parallels beta cell granula-

tion in other studies and one would expect from the insulin assays that beta cell granulation would be reduced The human material will vary greatly in the time after death at which the pancreas is taken for study and in the conditions under which it is kept This in itself may lead to changes in insulin content and in the granulation of the beta cells of the islets

*Stimulation of islet secretion* The factors involved in the stimulation of insulin production and secretion by islet cells are not clearly established Many consider that an elevation of the blood sugar level is the main stimulus for secretion Evidence in favor of this view was to be found in the observations that injections of sugar or hyperglycemic blood into the artery of a pancreas transplanted to the neck (188) or in its normal location (189) caused a fall in the blood sugar level, whereas the same amount of glucose injected elsewhere gave, if anything, a slight increase in blood sugar

Allen (190), working with *partially depancreatized* dogs, found that keeping the blood sugar at normal levels or below by the administration of phlorizin did not prevent the development of the degenerative changes in islet cells He concluded that the stimulus leading to hydropic degeneration was some humoral factor, not the excessively high blood sugar but possibly a *deficiency of its own secretion, insulin,* in the circulating blood More recently, Lukens, Dohan and Wolcott (191) observed that phlorizin treatment restored the blood glucose to normal levels and prevented the development of islet lesions in pituitary-injected dogs As a result of their findings they consider it likely that *hyperglycemia* is important in the pathogenesis of diabetes It is hard to believe that these differences in the effects obtained with phlorizin are due to the differences in the prepations used, but they are difficult to explain on any other basis

Woerner (192) maintained the blood sugar above the normal level in guinea pigs by the continuous intravenous injection of dextrose and found that there was first a reduction in the granulation of beta cells followed by an extensive increase in islet tissue Using larger amounts of sugar he found that the majority of the beta cells were exhausted or showed the beginning of degenerative changes (193) Houssay and associates (127), using a continuous intravenous infusion of glucose in dogs, kept the blood sugar for 4 days at levels approximating those obtained after pituitary injections None of the degenerative lesions were evident, the islets, on the contrary, showed signs of hyperplasia They concluded that the pituitary action on islet cells was not mediated by hyperglycemia Gomori, Friedman and Caldwell (194) observed that when the blood sugar was elevated in guinea pigs the specific beta cell granules disappeared and when the blood sugar returned to normal the beta cell granulation was restored

While the evidence for the influence of blood sugar level on insulin secretion is accepted generally, yet there is some indication that the blood insulin level may be involved It is impossible to say which of the two is the factor of prime importance or whether both are concerned in islet stimulation Blood sugar level and blood insulin level probably change at the same time but the rôle of blood insulin level cannot be assessed properly until better methods for measuring

insulin in the blood are available. Even when the blood insulin level can be measured accurately the problem still will not be simple because the level of insulin in blood will depend not only on the rate of insulin liberation by islet cells but also upon the rate of removal of insulin from the blood.

Some observers ascribe a large part in the regulation of insulin secretion to nervous factors. For a discussion of this aspect of the subject the reader is referred to publications by Houssay (195) (196) and Jensen (197). Houssay and his associates hold that the essential regulation of the secretion of insulin is by humoral factors, but that in the dog the nervous system does play a part, though a secondary and dispensable one.

*The rôle of the pituitary in the stimulation of insulin secretion is not definitely* established. It would appear that there is a fundamental control of islet activity which is independent of the pituitary gland. This normal control probably is a humoral one, though nervous factors may modify it somewhat. In addition to this fundamental regulation, a second mechanism is suggested by the results of the injections of anterior pituitary extracts. These seem to indicate that under certain experimental conditions a direct pituitary stimulation of the islets may occur. Whether or not this superimposed pituitary stimulation plays any part in the normal animal cannot be decided at present. It might be visualized as a special means for enhancing islet secretion when the need for insulin is greatly increased.

## SUMMARY

From the literature reviewed it is evident that insulin is produced in the pancreas by the beta cells of the islets of Langerhans and that the regulation of its level is a matter of some complexity. There are good methods available for its extraction, and the procedures for testing the potency of the extracts are satisfactory though not as precise as could be desired. The distribution of insulin is not uniform throughout the whole pancreas, hence small samples may give erroneous results. The manner in which the results of the assays are expressed is also very important.

The value for the insulin content of the pancreas in normal mammals is remarkably similar in different species, though the age of the animal and the diet taken will affect it. No definite seasonal change in insulin content has been established, but unaccountable fluctuations in level occur. Anesthesia with barbiturates does not greatly alter it. Infection, under some circumstances, has a questionable effect which may be the result of a diminished caloric intake. Ligation of the pancreatic ducts reduces the size of the pancreas and also the total amount of insulin present in that organ. Tumors of the islets usually contain relatively large amounts of insulin which may in some instances approximate the amount calculated to be present in *pure islet* tissue. In human diabetes the insulin content of the pancreas is low. This finding is similar to that obtained in experimental diabetes, for the content is greatly reduced after extensive partial pancreatectomy, administration of alloxan or injections of certain anterior pituitary extracts.

One of the most important factors affecting the insulin content of the pancreas is the diet The insulin content of pancreas in the rat is reduced by fasting, lessening the caloric intake of a balanced diet and by fat-feeding The reduction seems to be related to a deficiency of carbohydrate or carbohydrate-forming substances in the diet Diets rich in carbohydrate, on the other hand, do not appreciably increase the insulin content of the pancreas above the *normal* level The effect of feeding different diets after the insulin content has been reduced by fasting gives some indication that dietary factors other than carbohydrate may be involved in the regulation of the insulin content of the pancreas This conclusion is supported by the fact that diets deficient in certain amino acids and not adequate for growth or maintenance occasion an appreciable reduction in insulin content No specific effects of vitamins or of zinc feeding on the insulin content of pancreas have been established

The injection of large doses of insulin in the rat leads to a reduction in the insulin content of the pancreas and to depressive changes in the islets Insulin administration in fasting or fat-fed rats greatly augments the effect of these procedures, giving a still further reduction in insulin content

The insulin-reducing effect of fat-feeding in rats can still be obtained after removal of the gonads, adrenals or pituitary, and, in the adrenalectomized and hypophysectomized animal, the low content resulting from fat-feeding can be restored by giving a balanced diet The removal of these organs does not in itself lead to an appreciable change in the insulin content The findings indicate that normally there is a fundamental regulation of the insulin content of pancreas that does not involve the pituitary, adrenals or gonads

Injections of certain anterior pituitary preparations, however, lead to an increase in the insulin content of the pancreas in the rat Oestrogenic materials and thyroxin also have a similar action Some of the effects appear to be mediated through the pituitary gland In most instances when the insulin content of the pancreas is elevated there is some evidence that the treatment leads to an increase in islet volume The increase in insulin content therefore is presumably related to the increase in the volume of functioning beta cells

The administration of diabetogenic pituitary preparations or extensive partial pancreatectomy in the dog results in a very pronounced lowering of the insulin content of the pancreas accompanied by degranulation and hydropic degeneration of the beta cells of the islets of Langerhans

It is apparent that the insulin content of the pancreas may be reduced by two different and opposed groups of conditions One includes fasting, fat-feeding and the administration of insulin in the rat, the other takes in extensive partial pancreatectomy and the injection of diabetogenic anterior pituitary extracts in the dog The treatment with insulin helps to show up certain differences in the two groups Insulin injections enhance the effect of fasting and fat-feeding in the rat, reducing the insulin content of pancreas to very low values, whereas insulin tends to prevent the lowering of insulin content or the islet changes that would ordinarily result from pituitary injections and partial pancreatectomy The fact that fat-feeding and fasting also reduce the effect of these procedures

makes the contrast between the groups more striking The evidence seems to support the view that fasting, fat-feeding and insulin administration reduce the need for endogenous insulin and lower the insulin content of pancreas by making the islet cells less active In this group the lowering must result from a diminution in production relative to any change in liberation On the other hand, the great increase in islet stimulation resulting from the injection of anterior pituitary extracts or partial pancreatectomy probably leads to exhaustion of the beta cells through overwork The lowering of the insulin content of pancreas in this group would seem to result from an increase in the liberation of insulin that is out of proportion to any change in the production of that material

The factors primarily responsible for the stimulation of the beta cells of the islets are not clearly established Some contend that the blood sugar level plays the chief part in this regulation, though others believe that the blood insulin level may be involved The nervous system has only a minor rôle While the fundamental control of the insulin content of the pancreas is not dependent upon the pituitary adrenals or gonads, nevertheless, under certain experimental conditions, it would appear that a pituitary effect may be superimposed upon the essential mechanism It has not been demonstrated that such a direct pituitary influence operates in the normal animal, but if it does it would provide for an additional increase in the secretion of insulin when the need is great

The evidence indicates that, in experimental animals several means are available for reducing islet stimulation and lessening the strain on the beta cells There is reason to believe that the same procedures are effective also in humans While it is true that by their use islet damage can be prevented and the beta cells restored yet, on the other hand, their excessive application may lead to poor nutrition of the animal and, in some instances, to atrophy of the islets Since excessive "rest" may be undesirable as well as excessive stimulation, discretion must be used in the clinical application of these findings

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# PHYSIOLOGICAL ASPECTS OF HUMAN GENETICS, FIVE HUMAN BLOOD CHARACTERISTICS

HERLUF H STRANDSKOV

*Department of Zoology, The University of Chicago*

As new facts are uncovered relative to the genetics and physiology of human variations it becomes of interest to attempt to correlate and integrate them In this paper we have tried to do this for five human blood characteristics The five we have chosen for consideration are 1, the M N blood types, 2, the A B blood groups, 3, sickle cell anemia, 4, hemophilia, and 5, the Rh blood factor

*The M N blood types* In 1927 Landsteiner and Levine (72, 73) reported that human bloods fall into three types, depending upon the presence of one or both of two agglutinogens which they called M and N (These types are distinct from the better known A B groups ) An individual may possess only agglutinogen M in his red blood cells, only N, or both M and N No individual has ever been found to be lacking in both

The M N blood type of an individual is revealed by testing his blood with anti M and anti N sera produced in rabbits or in some other laboratory animal (For methods of technique, see 148, 118 ) Anti M and anti N agglutinins are normally not developed in human blood, that is, they are normally not isoagglutinins

In 1928 Landsteiner and Levine (74) announced that the M N blood variations are entirely genetically determined and that they are inherited in a relatively simple fashion According to them the observed M-N types result from variations in a single pair of autosomal alleles. (By a pair of alleles we mean a pair of genes occupying the same locus on a pair of homologous chromosomes. The term autosomal implies that the genes involved are not located on the sex chromosomes but on one of the other twenty three pairs of human chromosomes or autosomes as they are called ) According to the hypothesis suggested an individual develops only agglutinogen M when he is homozygous for one of the alleles, only N when he is homozygous for the other one, and both M and N when he is heterozygous Many later studies (see 148 and 11) have substantiated fully these conclusions.

When it has been definitely established that a set of alleles is involved in the determination of variations within a character it is conventional and extremely desirable to assign a common symbol to the locus of the alleles Generally an abbreviation of the character in question is chosen The different alleles are distinguished by capitalization or by sub- or superscript Landsteiner and Levine have never assigned any genetically appropriate symbols to the two alleles concerned in the determination of the M N blood types. In 1941 Strandskov (130) suggested $A^m$ and $A^n$ (A was chosen as an abbreviation of the term agglutinogen ) If we adopt these symbols the genotypes of the three M N blood types are

| BLOOD TYPE OR PHENOTYPE | GENOTYPE |
|---|---|
| M | $A^mA^m$ |
| MN | $A^mA^n$ |
| N | $A^nA^n$ |

On the basis of the information given above it can easily be shown that the offspring results expected from the various M-N matings are

| MATING | OFFSPRING RESULTS EXPECTED | |
|---|---|---|
| | Genotypic ratio | Phenotypic ratio |
| 1 $A^mA^m \times A^mA^m$ | *1 $A^mA^m$ | 1 M |
| 2 $A^mA^m \times A^mA^n$ | ½ $A^mA^m$ ½ $A^mA^n$ | ½ M ½ MN |
| 3 $A^mA^m \times A^nA^n$ | 1 $A^mA^n$ | 1 MN |
| 4 $A^mA^n \times A^mA^n$ | ¼ $A^mA^m$ ½ $A^mA^n$ ¼ $A^nA^n$ | ¼ M ½ MN ¼ N |
| 5 $A^mA^n \times A^nA^n$ | ½ $A^mA^n$ ½ $A^nA^n$ | ½ MN ½ N |
| 6 $A^nA^n \times A^nA^n$ | 1 $A^nA^n$ | 1 N |

* The coefficient 1 implies unity or all

The expected mating results presented above are obtained by applying the compound probability law of independent events For the calculation of expected results the form used in algebra is the most suitable

*Example*

Mating ♀ MN × ♂ MN or ♀ $A^mA^n \times$ ♂ $A^mA^n$  
eggs expected = ½ $A^m$ ½ $A^n$  
sperms " = ½ $A^m$ ½ $A^n$

¼ $A^mA^m$ ¼ $A^mA^n$  
¼ $A^mA^n$ ¼ $A^nA^n$

Genotypic ratio  
expected = ¼ $A^mA^m$ ½ $A^mA^n$ ¼ $A^nA^n$  
Phenotypic ratio  
expected = ¼ M ½ MN ¼ N

The $A^m$ and $A^n$ alleles have been shown to be inherited independently of the alleles which are responsible for the A-B blood group variations (6, 145)

According to the universally accepted tenets of genetics every cell of the body of a given individual possesses the same gene complex as did the zygote from which the individual developed Hence, for example, every cell of an MN individual possesses an $A^m$ and an $A^n$ gene From all indications, however, M and N agglutinogens are produced only within red blood cells At least Boyd and Boyd (12) and Wiener and Forer (149) were unable to detect M and N agglutinogens in tissues other than blood Boyd (10) even tested spermatozoa Hence it seems probable that only in red blood cells are environmental conditions favorable for the action of the $A^m$ and $A^n$ genes, at least in so far as the production of M and N agglutinogens is concerned The possibility exists that the $A^m$ and $A^n$ genes are responsible for the catalysis of physiological processes in other cells which produce other types of variations in those tissues, but so far no such

effects have been reported For a definition of a gene and a general discussion of gene physiology, see Wright (161)

We have already pointed out that every red blood cell of the heterozygote ($A^mA^n$) possesses both M and N agglutinogens This must mean that the two alleles $A^m$ and $A^n$ are responsible for the catalysis of somewhat independent chemical processes *This is a rather unusual condition Two alleles in a heterozygote* which is distinguishable from either homozygote generally produce an intermediate effect in one characteristic rather than two separate and clearly distinguishable characteristics such as the M and N agglutinogens.

Although both the M and the N agglutinogens are present in the heterozygote neither is as strongly developed in it as it is in its respective homozygote (73, 74, 154) This could mean that a single dose of each allele can not effect the catalysis of chemical processes to the same extent as two of them can when together Another possible explanation is that in the heterozygote both the $A^m$ and the $A^n$ alleles draw upon the same substrate and that there is an insufficiency of this material for a complete expression of each gene

Since the M and N agglutinogens are found only within red blood it seems probable that the effects of the $A^m$ and $A^n$ genes are entirely intracellular This is in contrast to many other human genes which produce extracellular effects through hormone systems or other cell products In turn the physiological activities of the M N alleles apparently are not influenced by hormone systems, at least not sex hormones, because we find the same M N phenotypic frequency among the two sexes

The $A^m$ and $A^n$ genes *apparently* are fully active prior to birth Moureau (98) has demonstrated the existence of M and N agglutinogens in the blood of human embryos as early as the second month of pre-natal life Hyman (66) found no changes in type following birth She believes that the type is fully established by the seventh month of intra uterine development if not earlier

It is not only possible to study the genetics of an individual but of a population as well This analysis consists in part, at least, of determining the relative frequencies of the alleles of every known gene locus. When a character is inherited in as simple a fashion as are the M N blood types it is actually possible to count the number of each allele within the population. For example, if it were found as a result of tests that a population in equilibrium consisted of 36,000 M, 48,000 MN and 16,000 N individuals, the relative frequencies of the $A^m$ and $A^n$ genes would be 60 per cent and 40 per cent respectively For any population the percentage frequency of the two M N alleles are

$$A^m = M + \frac{MN}{2}$$

$$A^n = N + \frac{MN}{2}$$

It is, of course, often impractical to test all individuals in a given population with respect to variations in an inherited character but sufficiently large samples for statistically reliable estimates are generally not too difficult to obtain

The frequencies of $A^m$ and $A^n$ genes have been determined for a large number

of populations Nearly every racial group has been examined It is not our intention to give here a complete list of such frequencies We shall present only the frequencies of three populations in table 1 for illustrative purposes (For extensive tables see 148, 11, 135 )

When gene frequencies with respect to a particular character have been determined for a given population it becomes of interest to attempt to account for them Presumably in the evolutionary history of man one member of each set of his alleles was the parental gene and gave rise by mutation to the other allele (or others) As regards the $A^m$ and $A^n$ alleles there is little evidence as to which came first Agglutinogens serologically similar to M and N have been found in the chimpanzee (73, 148, 23) Hence it seems probable that the origin of both the $A^m$ and the $A^n$ gene antedate man's origin Agglutinogens similar to M, but none similar to N, have been reported for orang-utans, gibbons and old world monkeys (22, 144) This suggests that the gene $A^m$ may be the older and the parent of $A^n$, but the evidence is not conclusive

TABLE 1

| POPULATION | INVESTIGATOR | NUMBER TESTED | PERCENTAGE FREQUENCY OF PHENOTYPES | | | PERCENTAGE FREQUENCY OF GENES | |
|---|---|---|---|---|---|---|---|
| | | | M | MN | N | $A^m$ | $A^n$ |
| U S whites, N Y City | Landsteiner and Levine | 532 | 26 1 | 53 6 | 20 3 | 52 9 | 47 1 |
| U S negroes, N Y City | Landsteiner and Levine | 181 | 27 6 | 47 5 | 24 9 | 51 35 | 48 65 |
| American Indians from Lawrence, Kansas | Landsteiner and Levine | 124 | 58 1 | 36 3 | 5 6 | 76 25 | 23 75 |

Even though we accept the suggestion that one of the M-N alleles has arisen by mutation from the other we have not accounted for the fact that $A^m$ and $A^n$ genes are nearly equally common in most human populations, nor have we accounted for the slightly different frequencies between different populations It must be obvious that if a single mutant gene appears in a large population it will immediately have only a very low frequency How then can it increase in proportion? There are three major ways in which this can happen These are *a*, recurrent mutation, *b*, accidents of sampling, and *c*, selection There is no evidence that recurrent mutations have occurred or are occurring within the M-N allelic set but this process could be going on at a fairly high rate without detection, because unexpected variations within M-N blood types are not easily recognized (Recurrent mutations in other human allelic sets have been reported (52, 53) ) Of course, if only one of the alleles mutated to the other the first would eventually become extinct The present day observed $A^m$ and $A^n$ frequencies could represent either an intermediate stage in a one way mutation system or an equilibrium or an approach toward an equilibrium in a system involving mutations in both directions (For a detailed discussion on theoretical

consequences of mutations and mutation rates in evolutionary systems see 157 158, 159, 38, 51)

If a population is small, accidents not related to survival value may accumulate and shift gene frequencies considerably (see 157, 159, 160, 162) Theoretically the shift should of course occur in both directions but conditions might be altered so that a change in one direction persisted. It is true that most present day intrabreeding human populations are large, but it is not improbable that the primate population which diverged in the direction of man was small and therefore provided an opportunity for an accidental increase in one or the other of the M-N alleles The observed differences in $A^m$ and $A^n$ gene frequencies between different present day human populations could possibly be explained entirely in terms of accidental shifts

Selection obviously may change gene frequencies in a population. There is however, no evidence that a differential survival value exists for M N alleles In fact, there is no evidence that the agglutinogens M and N serve any function whatsoever in the human system Of course the possibility that the $A^m$ and $A^n$ genes produce other effects which have a differential survival value must be allowed. (For a detailed discussion on the consequences of different selection pressures see 157, 158 159, 51, 38, 160, 162)

Since isoagglutinins for the M N agglutinogens are normally not developed within human individuals no consideration need be given to M N blood types in blood transfusions A knowledge of the genetics of M N blood types is, however, of considerable value in legal cases involving disputed parentage Tests can not prove that a given individual is the parent of a given child but they may prove that a given individual is not the parent A further value of a knowledge of the genetics of M N blood types in populations is to be found in its application to the diagnosis of twins and in problems relating to racial interrelationships

*The A B blood groups* The A B blood groups were discovered in 1901 by Landsteiner (69) As pointed out by him there are involved in these blood differences two isoagglutinogens A and B which are located in the red blood cells and two corresponding isoagglutinins a and b to be found in the blood plasma A given individual may possess both only one, or none of the isoagglutinogens If he has developed a given isoagglutinogen he lacks the corresponding isoagglutinin On the other hand if he lacks a given isoagglutinogen he possesses the corresponding isoagglutinin

The four A B blood groups are properly referred to as the Ab A, B and O groups For some time these groups were designated by Roman numerals but it so happened that two different sets of numbers were given them (the Moss and Jansky classification) This situation led to much confusion To clear up the dilemma the Health Committee of the League of Nations recommended for adoption the letter terminology originally suggested by von Dungern and Hirszfeld and mentioned above This is known as the International Nomenclature and is now the only system used in scientific publications

As is well known the four major A B blood groups have been shown to have a hereditary basis. Epstein and Ottenberg (37) were the first to find evidence of

this In 1910 von Dungern and Hirszfeld (33) concluded that two pairs of alleles were involved in their inheritance This two factor hypothesis, as it is called, was generally considered a correct genetic explanation until 1924 It gave expectations which agreed fairly closely with the observed data In 1924–25 Bernstein (4, 5) pointed out that three autosomal alleles in a population (that is three genes occupying a given locus on one of the autosomes) could also give four phenotypes corresponding to the four A-B groups Bernstein examined the results of many matings and found that they conformed closely with results expected on the basis of his triple allelomorph hypothesis In 1931 Strandskov (129) tested the two proposed hypotheses by the Chi Square method and found that the then available extensive blood group data agreed much more closely with the results expected on the basis of Bernstein's hypothesis than with results expected on the basis of the previously suggested two factor one Many other studies (see 127, 11, 148) have supported Bernstein Consequently his theory is now universally accepted with modifications as pointed out below

TABLE 2

| BLOOD GROUP | ISOAGGLUTINOGENS | | ISOAGGLUTININS | | GENOTYPES |
|---|---|---|---|---|---|
| AB | A | B | — | — | $I^AI^B$ |
| A | A | — | — | b | $I^AI^A$, $I^Ai$ |
| B | — | B | a | — | $I^BI^B$, $I^Bi$ |
| O | — | — | a | b | $ii$ |

Bernstein never assigned appropriate gene symbols to the three alleles which he considered to be involved In his 1931 paper Strandskov suggested $I^A$, $I^B$, and $i$ (The letter I was chosen as an abbreviation of the term isoagglutinogen )

In table 2 are shown the relationships of the isoagglutinogens and isoagglutinins within the four A-B blood groups, also shown are the genotypes according to Bernstein's triple allelomorph hypothesis

Although it is still correct to speak of four major A-B blood groups there is now conclusive evidence that at least one of the groups must be subdivided into two or more subgroups As early as 1910 von Dungern and Hirszfeld (33) found that when serum from certain group B individuals is mixed with certain group A bloods, until it loses the power to agglutinate the red cells of these A bloods, it still possesses the ability to agglutinate the red cells of other A bloods This suggested to them that there exist two kinds of A isoagglutinogens and two kinds of anti-A isoagglutinins That this is true has been substantiated by many later investigations In 1930 Landsteiner and Levine (77) designated the two A isoagglutinogens as $A_1$ and $A_2$ Accordingly group A is now subdivided into subgroup $A_1$ and $A_2$ and group AB into $A_1B$ and $A_2B$

Although two anti-A isoagglutinins apparently exist in both B and O bloods it has been found that they do not bear an exact one to one relationship with the $A_1$ and $A_2$ isoagglutinogens as might be expected It is true that one of the two existing isoagglutinins reacts mainly with $A_1$ bloods It has therefore been

termed $a_1$ The other, however, does not agglutinate only $A_2$ blood but reacts about equally well with $A_1$ It has therefore been termed the common anti A isoagglutinin and has been designated as a without a subscript. (A more appropriate symbol would seem to be $a_{1-2}$)

A third subgroup of A has been reported independently by Fischer and Hahn (39) and by Friedenreich (42, 41) but it apparently is relatively rare and difficult to detect. Subgroups of B have also been reported (91) but they also remain to be generally known

With the discovery of the $A_1$ and $A_2$ subgroups the question arose as to whether they also had a genetic basis In 1927 Landsteiner and Levine presented evidence that they do In 1930 Thomsen, Friedenreich and Worsaae (137, 138) pointed out that their inheritance could be explained by assuming a fourth allele in the series suggested originally by Bernstein That such a fourth allele exists has now been fully established (148, 11) Hence we now recognize alleles $I^{A_1}$, $I^{A_2}$, $I^B$ and i Additional alleles have been suggested for the other sub-

TABLE 3

| BLOOD GROUP OR PHENOTYPE | ISOAGGLUTINOGENS | ISOAGGLUTININS | GENOTYPE |
|---|---|---|---|
| $A_1B$ | $A_1$ B | — — — | $I^{A_1}I^B$ |
| $A_2B$ | $A_2$ B | — $a_1$ —* | $I^{A_2}I^B$ |
| $A_1$ | $A_1$ — | — — b | $I^{A_1}I^{A_1}$ $I^{A_1}I^{A_2}$ $I^{A_1}i$ |
| $A_2$ | $A_2$ — | — — b | $I^{A_2}I^{A_2}$ $I^{A_2}i$ |
| B | — B | a $a_1$ —† | $I^BI^B$ $I^Bi$ |
| O | none | a $a_1$ b | ii |

Isoagglutinin $a_1$ is only rarely found in $A_2B$ bloods

† Both a and $a_1$ are present in all B and O bloods but their titers vary considerably

groups which have been observed but since they do not appear to be clearly established as yet, we may omit a discussion of them here

The relationships of the isoagglutinogens and isoagglutinins within the four major groups and their subgroups are shown in table 3 also shown are the genotypes of each group

The relationships of the various genotypes to the six definitely established blood groups deserve some discussion It may be seen that both the $A_1$ and B isoagglutinogens are formed in the red blood cells of the heterozygote $I^{A_1}I^B$ This must mean that neither of these two alleles is dominant over the other and also that they catalyze distinctly different chemical reactions within the same cells. The same relationships hold for the $I^{A_2}$ and $I^B$ alleles As we pointed out in connection with the genes responsible for the production of the M N agglutinogens it is rather unusual for two alleles to produce two distinctly different characteristics in the heterozygote. The $I^{A_1}$, $I^{A_2}$ and $I^B$ alleles, as might be expected, produce only their respective isoagglutinogens when present in the homozygous condition According to Thomsen, Friedenreich and Worsaae (137, 138) the $I^{A_1}$ allele is completely dominant over the $I^{A_2}$ allele so that only

isoagglutinogen $A_1$ is produced in the heterozygote $I^{A_1}I^{A_2}$ The gene $I^{A_1}$ apparently is also completely dominant over i This last statement has physiological meaning because the gene i has been shown to produce an isoagglutinogen when homozygous At least an isoagglutinin (anti O) has been found in some $A_1$ and $A_1B$ bloods which agglutinates all group O bloods (76, 136) The $I^{A_2}$ and $I^B$ alleles are probably not completely dominant over i because some $A_2$ and some B bloods give slight reactions with anti-O sera These presumably are those of the heterozygotes $I^{A_2}i$ and $I^Bi$ (136)

Little or no information is available relative to the question of whether a single dose of each of the $I^{A_1}$, $I^{A_2}$ and $I^B$ genes produces as much isoagglutinogen as do two of them in the homozygous condition

A-B alleles apparently are functional within nearly all cells of the body At least A-B group specific substances have been found in most body tissues (71, 148, 11) An exception is the fetal part of the placenta (114, 101) Their absence in that organ may be an evolutionary adaptation which prevented reactions between fetus and mother Schiff and Weiler (121) have postulated an enzyme in the placenta which destroys the A-B substances which they assume to be produced there A-B substances have also been found in most body fluids and gland secretions (82, 111, 148, 118) They are abundantly present in saliva and gastric juice

An interesting variation has been found with respect to the presence or absence of A and B group specific substances in the saliva and other secretions In some individuals these substances may be present in high concentrations (secretors), whereas in others (non-secretors) they are absent or nearly absent (82, 111, 148, 118) Schiff and Sasaki (119, 120) were able to demonstrate that these variations have a hereditary basis and that they are dependent upon variations at a single autosomal locus The allele for the ability to secrete (S) is dominant over that for non-secretion (s) The secretor alleles are inherited independently of those for the production of A-B isoagglutinogens

So far we have not considered the causes of the production of the specific isoagglutinins which are present in the various A-B blood groups There appear to be several possible but unsubstantiated explanations for their existence One is that the same alleles which are responsible for the formation of the isoagglutinogens are also responsible for the isoagglutinins (44) This is a rather attractive hypothesis because of the consistency between the A-B genotypes and the isoagglutinin formed Difficulties, however, arise when one attempts to consider the physiological relationships involved What reason would there be for a given gene to produce a particular antigen and also a set of antibodies for antigens produced by other genes belonging to its allelic series? That a given gene will not produce an antibody for the particular antigen it develops is logical but why should it be responsible for the development of antibodies for other antigens within the same species? A second possible explanation (117) is that the A-B alleles are responsible only for the A-B isoagglutinogens but that these in turn initiate the formation of the isoagglutinins It is possible to imagine that one allele produces small quantities of one isoagglutinogen, whereas

another produces large quantities of it. Furthermore one can imagine that when small quantities of an isoagglutinogen are formed the corresponding antibodies destroy them, whereas when large quantities of an isoagglutinogen are formed the corresponding antibodies are absorbed. Although this hypothesis has certain attractive features it does not fit in well with Bernstein's triple allelomorph hypothesis. It will be recalled that according to this hypothesis individuals belonging to blood group O are homozygous ii and should therefore produce only one kind of antigen These individuals, however, develop three kinds of antibodies, namely, the common anti A agglutinin, anti-$A_2$, and anti B A third possible hypothesis is that the A B alleles are responsible only for the formation of A-B isoagglutinogens and that the corresponding isoagglutinins are formed as a result of other genetic factors This hypothesis assumes that all the A B isoagglutinins are formed in all human individuals but that in the presence of a particular antigen the corresponding antibody is absorbed Bernstein has favored this hypothesis and to the author also it seems the most probable A possible variant is that each gene which is responsible for the formation of a particular antigen inhibits at the same time the formation of the corresponding antibody An allele which produced such an inhibition effect would have been strongly selected for in the course of evolution.

If we accept the assumption of this last mentioned hypothesis, namely, that the A B isoagglutinins are produced normally, i e , due to other genetic factors, we must account for the existence of such genetic factors. One possible explanation is that the A B isoagglutinogens are similar to other antigens which are commonly found in lower forms which parasitized man's ancestors. There is some evidence that A B group specific substances are similar to such commonly found antigens For example, it has been shown that agglutinogen A resembles the Forsman antigen which is found in many bacteria as well as in many other parasitic forms (70, 27, 35 116, 43)

Human populations have also been studied with respect to the distribution of the A B alleles The formulae for the determination of the A B gene frequencies from observed phenotypic frequencies are somewhat more complicated than those for the M N alleles, but they are not difficult to apply If we assume random mating in a population (and there is every reason to believe that we may) these formulae for the frequencies of the four alleles are obtained from the square of the frequency array of the four alleles ($I^{A_1} + I^{A_2} + I^B, + i$) or the square of ($p_1 + p_2 + q + r$) By taking into account the genotypic and phenotypic relationships, the frequencies of the four alleles in any population, based on the empirically determined A B phenotypic frequencies are as follows

$$
\begin{aligned}
p_1 &= \sqrt{A_1 + A_2 + O} - \sqrt{A_2 + O} \\
p_2 &= \sqrt{A_2 + O} - \sqrt{O} \\
q &= \sqrt{B + O} - \sqrt{O} \\
r &= \sqrt{O} \text{ or } 1 - (p_1 + p_2 + q)
\end{aligned}
$$

Since the fourth allele of the A-B series has been recognized only a relatively short time not many population studies have included it, but the frequencies

with respect to the three originally recognized alleles have been determined for many populations (11, 127)  A few sample frequencies including all four alleles are given in table 4

As suggested by table 4 the A-B gene frequencies vary considerably among different human populations  All four alleles are, however, represented in all or at least in nearly all populations  This suggests that all four alleles were present in the original human stock  This point of view is supported by the fact that agglutinogens serologically similar to the human A-B antigens have been found among Anthropoid apes and lower primate groups (34, 78, 3, 6)  There is little or no evidence as to which of the four alleles appeared first in the course of evolution and there is little or no evidence on the basis of which to account for the present day A-B gene frequencies  Mutations within the A-B allelic series have not been reported but again this is not surprising in view of the fact that such mutations are extremely difficult to detect  Selection may have played a rôle in decreasing or increasing a given gene's frequency within a particular population but actually there is no conclusive evidence that any of the A-B alleles have a greater or a lesser survival value than any of the others  Accidents of sampling could account for some of the frequency differences between populations, particularly differences between small populations such are found among some of the North American Indian tribes

TABLE 4

| POPULATION | INVESTIGATOR | NUMBER TESTED | PERCENTAGE FREQUENCY OF GROUPS | | | | | | PERCENTAGE FREQUENCY OF GENES | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $A_1B$ | $A_2B$ | $A_1$ | $A_2$ | B | O | $I^{A_1}$ | $I^{A_2}$ | $I^B$ | i |
| U S whites | Wiener and Sonn | 1077 | 5 2 | 1 4 | 29 0 | 8 9 | 13 9 | 41 7 | 18 1 | 6 5 | 10 0 | 64 6 |
| U S negroes | Wiener | 189 | 1 6 | 1 1 | 19 6 | 6 8 | 22 8 | 48 1 | 12 2 | 4 8 | 14 5 | 69 4 |
| Full-blooded American Indians | Landsteiner, Wiener and Matson | 120 | 0 | 0 | 25 8 | 0 | 0 8 | 73 3 | 13 9 | 0 0 | 0 5 | 85 6 |

In contrast to the M-N blood types the A-B blood groups must be considered in blood transfusions  Some consider group AB individuals universal recipients and group O individuals universal donors  It is, however, advisable to use, except in emergencies, only donors of the same blood group as the host  In addition to their importance in blood transfusions A-B blood group determinations are exceedingly valuable in the solution of cases of disputed parentage  However, as was true for the M-N blood types no individual on the basis of A-B determinations can be proven to be the parent of a given child  He may only be shown not to be the parent  A-B blood group determinations have also wide application in the diagnosis of twins and in problems relating to racial origins

*Sickle cell anemia*  Sickle cell anemia was first reported by Herrick (61) in 1910  The patient was a negro youth from the West Indies  Since this first report many similar cases have been found  The diagnostic feature is the presence of crescentic or sickle shaped red blood cells  These may not be common

in the blood smear but susceptible cells can be induced to sickle by sealing them under a cover slip with petrolatum and incubating them at room temperature for twenty four hours (70) or by a number of other techniques (65, 50, 132, 2, 32)

In 1923 Huck (65) and Taliaferro and Huck (134) presented evidence that sickle cell anemia is a familial disease and that it is inherited as an autosomal dominant According to this hypothesis all affected individuals possess at least one dominant gene (Si) and all normal individuals are homozygous recessive (si si) Although only a limited number of studies have been carried out to test this mode of inheritance, the combined genetic evidence obtained since then (49, 96, 99, 115) seems to substantiate it

As we mentioned above, the most striking physiological effect of the Si gene is the production of a condition within red blood cells which makes them susceptible to sickling What this condition is no one has discovered as yet There seems no question but what the effect resides within the red blood cells because Huck (65) and Sydenstricker (132) have shown that the red cells of sickle cell patients sickle when washed with serum from normal persons, whereas the serum from the former does not cause cells of the latter to sickle Emmel (36) has suggested that the sickling may be only an accentuation of the normal process which causes red blood cells to assume the biconcave disk shape.

According to Sydenstricker (132) and Emmel (36) sickled cells are immediately subject to phagocytosis. Large mononuclear cells occur in the blood of sickle cell patients which phagocytose sickled cells but will not attack the red cells of normal blood Wollstein and Kreedel (141) report that the Kupfer cells are active in the removal of sickled cells. Cardozo (17) has tested for the presence of specific agglutinogens but has failed to find such substances. Hahn and Gillespie (49) have reported that susceptible cells can be induced to sickle in an atmosphere of carbon dioxide and caused to revert to normal shape when saturated with oxygen However, Hein McCalla and Thorne (60) and Graham and McCarty (45) did not find increased sickling upon placing susceptible cells in closed chambers.

The Si gene apparently produces its effect early in the development of red blood cells Cooley and Lee (19) have reported sickled cells that were nucleated and Jaffe (67) and others have observed reticulated cells

Whether all the red cells of all sickle cell patients are subject to sickling is not certain Variations occur in the number which respond Anderson and Ware (1) generalize by saying "Ninety per cent of the supply of new cells are sickle cells" This, however, does not necessarily mean that the rest are not susceptible

Other conditions which have been reported to be associated with sickling may be secondary effects of the Si gene rather than primary ones. Anderson and Ware (1) outline secondary effects as follows

When they (the red cells of sickle cell patients) are put into circulation all except about 2% of the sickle cells are destroyed by phagocytosis This brings about a greater activity of the bone marrow in the production of new cells as is indicated by the increase in reticulocytes The spleen and liver enlarge to take care of the influx of young cells and a vicious

cycle is established As fast as the red cells form they become sickle cells and are phagocytosed The bone marrow cannot continue its hyperactivity indefinitely, and as the disease progresses, fewer new cells are put into circulation The stimulus that caused the spleen to enlarge is thus removed and it decreases in size The liver, however, remains enlarged, possibly because its various other functions make it less responsive to the failure of the haematopoietic system

Some investigators (95, 139, 13, 29, 47, 59) have reported bone changes, particularly in the skull and long bones Cardozo (17), however, found no such changes in 17 patients which he studied specifically for such effects With respect to other associated conditions Anderson and Ware (1) write

> The patient usually gives a history of having been weak and sickly for a number of years and of having previous attacks of weakness, jaundice, fever and abdominal or articular pain There is usually a history of repeated acute infection of the respiratory tract The course of such infection is usually longer than that in normal persons

Recently Wertham, Mitchell and Angrist (143) have reported changes in the central nervous system They find "focal and diffuse changes in the nerve cells in cortical and sub-cortical gray structures, and focal areas of demyelination in the spinal cord"

For a long time it was thought that sickle cell anemia was limited to the negroid stock However, in 1929 Cooley and Lee (20) reported a case in a Greek child which could not reasonably be attributed to race admixture Since then a number of other instances of sickling have been found in non-negroid populations (125, 115, 18, 108, 140, 46, 96, 99) Most of these cases are from descendants of Southern European peoples Ogden (99) examined 1,602 unselected, consecutive patients which included 692 negroes and 910 whites Among the negroes he found 45 who showed the sickling trait, whereas among the 910 whites he found none Ogden writes in 1943 as follows

> I believe I have a right to my strong conviction that the sickling trait is a condition found in the negro race only and that in all cases in which members of white families have such a trait an admixture of negro blood in the immediate or remote ancestry has taken place In no case of the sickling trait in a white person reported up to the present time has the possibility of negro blood been excluded

One must admit the possibility of Ogden's contention, but it does seem unlikely that all cases reported among Caucasoids are to be explained in terms of race admixture

Estimates of the incidence of sickle cell anemia among negroes range from 4 3 per cent (133) to 9 42 per cent (17) Ogden (99) obtained a frequency of 6 5 per cent among 692 unselected negroes Cardozo (17) combined all published data and arrived at a frequency of 7 44 per cent This percentage is based on an examination by different investigators of 11,021 individuals

A difference in sex incidence has been reported which may be significant Cardozo (17) finds that 69 1 per cent of those showing sickling are females Ogden (99) in his series found 37 females to 8 males If this sex difference is a real one there has yet been no explanation advanced to account for it An

autosomal dominant characteristic should occur with equal frequency among females and males

It is of interest to estimate the incidence of the Si and si genes in populations If a trait due to an autosomal dominant gene such as Si has a frequency of 7 44 per cent in a population in which mating is occurring at random the recessive character (in this case the normal condition) obviously has an incidence of 92 56 per cent. The best estimate of an autosomal recessive gene in a population mating at random is obtained by extracting the square root of the incidence of the recessive character in the population Therefore, it may be estimated that the si gene in negroid populations has a frequency equal to square root of 92.56 or 96.2 per cent. It follows, of course, if only two alleles exist that the dominant gene has an incidence of 3 8 per cent What the frequency of the Si gene is in Caucasoid and Mongoloid populations can not be determined until more data are available Perhaps as Ogden insists it does not occur at all in these groups except as the result of race admixture

As must be obvious from the symptoms associated with sickle cell anemia selection against the Si gene must be fairly strong Anderson and Ware (1) write "Owing to his decreased resistance he (the patient) is a prey to the various infectious diseases and usually succumbs to one of these. Few patients live beyond the age of 35 years' If selection operates as strongly as this statement suggests it becomes a problem to account for the Si gene's high incidence among negroes One possible explanation is that the si gene mutates to the Si gene at a high rate within this group There is, however, no direct evidence that such mutations are taking place, but it seems almost inevitable that they must be. The lower incidence of the Si gene among Mongoloids and Causasoids could be accounted for either in terms of a lower mutation rate within these groups or in terms of a higher selection pressure

A knowledge of the genetics of sickle cell anemia has only a limited application to forensic medicine but such knowledge does contribute to our prediction of the occurrence of sickle cell anemia in a family and therefore to our chances of discovering cases in their incipient stages Studies on the frequency of the Si and si alleles in different populations can contribute to the solution of problems relating to racial interrelationships.

*Hemophilia* The earliest unmistakable case of hemophilia was reported in the sixteenth century by Albucasis However, a clear and concise description was first given in 1803 by Dr John C Otto of Philadelphia (102) He was also the first to point out that hemophilia has a hereditary basis, a suggestion which has been fully substantiated Otto did not suggest any specific Mendelian mode of inheritance This is not surprising in view of the fact that his publication antedated Mendel's original discovery by more than sixty years Otto did nevertheless, present the view that only males show the condition and that they inherit it through unaffected females

When sex-linked inheritance was discovered in the fruit fly, Drosophila melanogaster, about 1910 it became apparent that hemophilia in man was probably inherited as a sex linked recessive (97) This implies that the hemophilic gene

(h) and its normal allele (H) have their common locus on the X chromosome, and that a single hemophilic gene (h) will produce the condition in the male, whereas in the female two are necessary for its expression Nearly all the published pedigrees support this mode of inheritance Haldane (55) has presented evidence of linkage between the locus of the hemophilic gene and that for red-green color blindness which is known to be sex-linked This is confirmatory evidence for sex-linkage After Haldane (52) had published evidence that some other genes of man are partially sex-linked, i e , carried on the region of the X-chromosome where crossing over occurs with the Y-chromosome, Sirks (126) suggested that the hemophilic gene might also belong in this category However, as Haldane (58) has already pointed out, Sirk's arguments are not very convincing A few pedigrees, particularly those of Bess Lloyd (88), suggest that the hemophilic gene, although sex-linked, may not always be completely recessive She reports four females who were heterozygous and gave evidence of being bleeders She states "they generally are not as severely affected as the males " Warde (142) also reports a female as hemophilic who probably was only heterozygous Her father and only son were hemophilics but her mother had no hemophilic ancestry Foulis and Crawford (40) reported two female bleeders in a hemophilic pedigree Their fathers were normal but one of the females had two hemophilic sons which is evidence that she at least was heterozygous, i e , a carrier Foulis and Crawford admit that their two cases might represent purpura hemorrhagica rather than true hemophilia, but there at least is a suggestion that the effect was due to the hemophilic gene Although the few pedigrees we have mentioned suggest incomplete recessiveness on the part of the hemophilic gene most heterozygous females show no effect Hence it still seems justifiable to conclude that the common form of hemophilia is not only sex-linked but completely recessive The heterozygous females which show a condition similar to hemophilia may possess a variant of the common allele or present a clinical picture similar to hemophilia due to other factors

If the gene for hemophilia (h) and its normal allele (H) are sex-linked the expected genotypic results of the six possible matings are as follows

1 ♀ HH x ♂ H(y) = $\frac{1}{2}$ ♀ HH $\frac{1}{2}$ ♂ H(y)
2 ♀ Hh x ♂ H(y) = $\frac{1}{4}$ ♀ HH $\frac{1}{4}$ ♀ Hh $\frac{1}{4}$ ♂ H(y) $\frac{1}{4}$ ♂ h(y)
3 ♀ hh x ♂ H(y) = $\frac{1}{2}$ ♀ Hh $\frac{1}{2}$ ♂ h(y)
4 ♀ HH x ♂ h(y) = $\frac{1}{2}$ ♀ Hh $\frac{1}{2}$ ♂ H(y)
5 ♀ Hh x ♂ h(y) = $\frac{1}{4}$ ♀ Hh $\frac{1}{4}$ ♀ hh $\frac{1}{4}$ ♂ H(y) $\frac{1}{4}$ ♂ h(y)
6 ♀ hh x ♂ h(y) = $\frac{1}{2}$ ♀ hh $\frac{1}{2}$ ♂ h(y)

The expected results are calculated as shown below Mating no 5 is used as an illustration

Mating ♀ Hh x ♂ h(y)
eggs expected = $\frac{1}{2}$ H $\frac{1}{2}$ h
sperm expected = $\frac{1}{2}$ h $\frac{1}{2}$ (y)
Genotypic ratio ————————————
expected = $\frac{1}{4}$ ♀ Hh $\frac{1}{4}$ ♀ hh $\frac{1}{4}$ ♂ H(y) $\frac{1}{4}$ ♂ h(y)

One of the problems relative to hemophilia that has puzzled many people is

the question of why so few females are hemophilics (if any are), when so many males are afflicted Why this sex difference in incidence? At least a partial answer to this question is to be found in the manner in which hemophilia is inherited If a character is inherited as a sex linked recessive, and is relatively rare in a population which is mating at random, it should theoretically be expected to be much more common among males than females Let us assume for instance that a pair of sex-linked alleles, B and b, have frequencies in a population of 90 per cent and 10 per cent, respectively Since only a single gene is necessary to express the character in a male these should show the gene frequency directly, i e., 90 per cent of the males should show the dominant sex linked character due to (B) and 10 per cent should show the recessive due to b On the other hand in the female two recessive genes (bb) must come together to show the recessive character Hence the frequency of a sex linked recessive character expected among females is only the square of the recessive gene frequency or in the above case 1 per cent (The probability of two independent events occurring in combination is the product of their independent chances ) The expected difference in sex incidence can be made a little more striking by resort to another example If for instance a sex linked recessive character has an incidence of 1 in 100,000 among males we should theoretically expect only 1 female among 10,000,000,000 to show it The incidence of hemophilia among males is not as low as 1 in 100 000 but it is low enough to give a decidedly large expected sex difference What the exact incidence of hemophilia is in most human populations has not been determined with any degree of accuracy Haldane (55) has estimated for the population of London an incidence of about 1 in 10,000 among males If we accept this figure for every population we should expect only 1 female among every 100 000,000 to be homozygous hh and therefore hemophilic

Other factors make the occurrence of hemophilia among females even more improbable In order to produce a homozygous female (hh) it is necessary that her father be a hemophilic and her mother homozygous (hh) or a carrier (Hh) In the latter type of mating a homozygous female (hh) has only a probability of 50 per cent. Now it is well known that hemophilic males tend to die early Estimates have indicated that 54 per cent of hemophilic males die before the fifth year and that 88 per cent die before the twentieth Snyder (128) states that only 11 per cent of hemophilic males live to be 22 Thus there is a strong chance that hemophilic males will not marry because of death Furthermore of those who live relatively few marry Snyder (128) examined 250 of the published pedigrees on hemophilia and found only one instance where a known hemophilic male had married a known carrier female and where a hemophilic daughter might therefore be expected This was pedigree no 407 in the collection published in 1911 by Bullock and Fildes (16) This mating produced one hemophilic son and one normal daughter Thus from this mating a homozygous female (hh) was probably not even realized

Some authors have postulated that the hemophilic gene produces a lethal effect early in life when it is present in a double dose in the female Others such as Birch (9) have suggested that the homozygous female (hh) survives but fails to

show the hemophilic condition due to alleviating effects produced by female hormones Although these supplementary hypotheses may be correct and may explain in part the complete or nearly complete absence of hemophilia among females they do not seem to be necessary The probability of a homozygous (hh) female in a given population is practically nil

Physiologically the hemophilic gene (h) produces interesting and serious effects The literature on this subject is generally known and has been frequently summarized It therefore seems unnecessary to review it here (See 122, 64, 3, 8, 93, 105, 104, 94, 63, 90, 26, 124, 123, 62, 14, 24, 28, 48, 89, 106, 112 )

Hemophilia probably occurs in all racial stocks Its incidence among Caucasoids, as pointed out above, is not accurately determined for any given population, but estimates have been made for a few of them Haldane (55) believes that among male births in London the incidence of hemophilia lies somewhere between 35 and 175 per million Bullock and Fildes (16) think that hemophilia is a good deal more common in Northern than in Southern Europe They do not give specific data Komai (68) is of the opinion that the same type of hemophilia which appears in the Caucasoid stock occurs in the Mongoloid He presents a number of pedigrees which agree with a sex-linked recessive mode of inheritance He does not give an estimate of its frequency among Mongoloids Among Negroids hemophilia is practically unknown Bullock and Fildes (16) found three cases reported among negroes, but they did not consider any of them reliable In 1936 Crandall (21) reported hemophilia in a full blooded negro and he indicated at that time that he believed his patient to be the only authentic case among negroes The genealogy according to Crandall did not, however, give evidence of inheritance In 1937 Pachman (103) reported three cases of hemophilia among negroes which had been seen at Duke Hospital According to Pachman "Two of them have a definite family history and a genealogy which is fairly typical In the third case, the diagnosis may be questioned but the history and x-ray findings are typical of hemophilia " He writes further "the skin and features of cases 1 and 3 were those of a full blooded negro, but in case 2 the color was lighter indicating that a mixture may have occurred "

An interesting problem from a genetic point of view is the question of how the frequency of the hemophilic gene is maintained at as high a level as it is in the face of the selection pressure which it encounters As mentioned earlier most hemophilic males fail to marry and consequently to leave offspring Under these conditions alone the (h) gene should gradually be reduced in frequency or eliminated completely To account for what appears to be a nearly constant frequency for this gene Haldane (52, 55) has suggested that mutations from H to h are taking place He estimates that one mutation occurs per 50,000 individuals per generation Enough sporadic cases of hemophilia have been reported to give credence to this hypothesis No good explanation is available to account for racial differences in incidence

A knowledge of the mode of inheritance of hemophilia is only of limited value in forensic medicine, but it is of importance to a practising physician in his determination of the probability of its occurrence in a given family To the human

geneticist hemophilia is an extremely interesting characteristic It offers unusual opportunities for an analysis of certain aspects of all the various factors which operate in the evolution of man

*The Rh blood factor* In 1940 Landsteiner and Wiener (79) discovered a new blood agglutinogen It was called Rh because it was revealed as the result of using serum from rabbits into which rhesus monkey blood had been introduced An interesting discovery with respect to it was that only about 86 per cent of the human white population gave positive reactions (Rh+) to the anti Rh serum while the remaining 14 per cent gave negative reactions (Rh−) The following year the same two investigators (81) tested for the rôle of heredity They examined 60 families which included 237 children They concluded that the variations have a hereditary basis and that only a single pair of autosomal alleles is involved They found that an individual is Rh+ if he possesses at least one dominant gene (Rh) and Rh− when homozygous recessive (rh rh) In a later paper Wiener and Sonn (140) extended this genetic study to include 40 additional families with 138 children Their results substantiated the previously suggested genetic hypothesis

In the last year or two evidence has been obtained (146, 150 84, 83) which suggests that at least 5 different Rh agglutinogens exist According to Wiener (147) these may occur in different combinations in different individuals and give 8 Rh blood types (including the Rh negative type) The different Rh agglutinogens give different presence and absence frequencies within the same populations In 1943 Wiener and Landsteiner (150) presented evidence that some of these new Rh subdivisions have a hereditary basis, and that at least three alleles are involved instead of two as was originally suggested More recently Wiener (147) has postulated 6 alleles Although Wiener has presented some evidence for the existence of 6 alleles it seems too early to consider all of them fully established

Although Rh alleles seemingly produce direct effects only within red blood cells, there is considerable evidence to suggest that they may be responsible for rather serious secondary effects. In 1939 Levine and Stetson (87) reported a fatal hemolytic reaction in a woman who had been given blood transfusion following a stillbirth The transfused blood, according to the usual tests, was compatible with the blood of the host. Yet a reaction occurred. The authors postulated that the fetus had inherited from its father the capacity to develop certain antigens which were not present in the mother and which induced the formation of antibodies in her tissues Further cases of transfusion reactions which could be attributed to iso-immunization following pregnancy were reported in 1940 by Levine and Katzin (85) The same year Wiener and Peters (151) pointed out that every recorded case of severe hemolytic reactions following first transfusions had occurred in post partum patients In 1941, following the discovery of the Rh factor and its inheritance, Levine et al (86) expressed the belief that several cases of miscarriages, stillbirths and of erythroblastosis fetalis which they had encountered were probably attributable to the Rh factor They postulated that these effects might result when the mother was Rh− and the fetus was Rh+, due to its heredity from the paternal side. They postulated that Rh

antigens of the fetus had entered the maternal blood stream, caused the formation of antibodies in her tissues and that these in turn had filtered through the placenta and reacted with the Rh+ antigen in the fetus It is of interest that Ottenberg (100) and Darrow (25) had previously suggested a serological explanation for erythroblastosis but had not been able to demonstrate any incompatibility between the blood of the fetus and that of the mother (For a clinical description and diagnosis of erythroblastosis fetalis see (31, 30, 7, 109, 107, 92, 15) )

That most if not all cases of erythroblastosis fetalis may be attributed to Rh blood factors seems probable Potter, Davidsohn and Crunden (110) found that among 60 mothers who had given birth to babies which had been diagnosed as erythroblastotic 90 per cent were Rh negative The infants which had been diagnosed as erythroblastotic and born to Rh+ mothers, these investigators believe, might actually be suffering from "a different disease entity" Another possible explanation is that each of these mothers was negative with respect to an Rh agglutinogen other than the one tested for, whereas the fetus was positive with respect to it

Although erythroblastosis may result when the mother is Rh− and the fetus is Rh+ it does not follow that it invariably does The fact is that the disease occurs in only a small percentage of cases where this relationship exists What the placental peculiarity is which results in the occurrence or absence of erythroblastosis is not known It is known that if the disease has occurred once in a family it is apt to recur This may mean that some hereditary basis exists for this placental peculiarity

Recently Witebsky and Heide (155, 156) have reported the occurrence of Rh antibodies in the breast milk of mothers who are Rh− and whose children are Rh+ and erythroblastotic These investigators believe that such antibodies might do further damage to the red blood cells of erythroblastotic children who consume such milk

From the population genetics point of view the Rh factor gives promise of being extremely interesting As we have already indicated the different Rh agglutinogens have different frequencies within the white population This means that the various alleles which are responsible for them also have different frequencies within that group In 1942 Landsteiner, Wiener and Matson (81) reported on the blood of 120 full blooded American Indians and 155 of mixed ancestry Among the 120 full blooded Indians they found only a single individual who was Rh− Among the 155 mixed bloods they found an incidence intermediate between that for full blooded Indians and whites More recently Wiener, Sonn and Belkin (153) have tested Chinese and Negro populations They found the Chinese like the American Indians to have a very low Rh− frequency Type Rh, which is a subdivision of the original Rh+ group, and which is rare in the white population, they found to have a low incidence among Chinese but to be very common among negroes

With the number of Rh alleles unsettled it is too early to discuss in detail the possible rôles of the various evolutionary factors in the determination of Rh

gene frequencies in populations It is, however, of interest to call attention to the manner in which selection operates As Haldane (58) has pointed out, selection is against the heterozygote Therefore an Rh and an rh gene is eliminated every time selection operates If positive and negative producing Rh alleles were equally common in the population neither of the two types of alleles would be changed in frequency The negative allele, however, is less common, therefore there is a tendency for it to be decreased in proportion to the positive alleles. The rate at which it is being decreased is, however, a very slow one Haldane (58) has estimated that it would require 619 generations or about 15,000 years to reduce the frequency of homozygous recessive individuals (rh rh) from its present figure, which is about 14 per cent, down to 1 per cent This is based on the assumption that no mutations to the rh allele are occurring There is no evidence that there are For the rh allele to be eliminated completely, without intervention by man, many more generations would be required

The question is frequently asked if it would not be desirable to advocate laws to prevent rh rh females from marrying Rh+ males Although such a law would probably eliminate the occurrence of erythroblastosis fetalis there is no justification for such an extreme measure. The incidence of erythroblastosis from such marriages is not high enough to warrant it A more reasonable position is taken by Haldane (58) He writes "Even if no systematic attempt were made to eradicate such a gene there would be a strong case for dissuading rh rh women known to be so constituted as to be destined to form permeable placentae from marrying Rh Rh or Rh rh men" This suggestion seems defensible

Since iso-immunization to Rh agglutinogens is possible Rh blood types should be considered in blood transfusions, especially if repeated transfusions are to be given

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# VERTEBRATE SMOOTH MUSCLE

ERNST FISCHER

*Department of Physiology and Department of Physical Medicine Medical College of Virginia Richmond*

Our present knowledge of smooth muscle is nearly as vague as it was when this subject was last reviewed in this journal Although 18 years of intensive research have passed in the meantime, the introductory remarks of Evans (81) about the reasons for this lack of precise knowledge could be repeated literally today The main change in our general understanding of "smooth muscle" is the deepened recognition that the conception of "smooth muscle" as a biological unity is completely misleading This is true from various points of view We can regard "striated muscle" of vertebrates as a unity since with negligible exceptions, histological, physiological and pharmacological differences are small, not only between the different anatomical muscles of the same animal but also between muscles from representatives of all five classes of vertebrates. For smooth muscles important differences exist, not only between the various smooth muscle tissues of one animal, but also between anatomically and functionally comparable smooth muscles even of related species Striated muscles are more or less all organs with the same functions and consist practically of muscle tissue alone Therefore, they can be easily subdivided into units, motor units, or even single striated fibers, all of which have to a certain extent the same physiological properties as the whole anatomical muscle. As a rule, smooth muscles are only elements participating with various other tissues in forming organs of quite different functions such as stomach, artery, spleen, uterus, iris, etc If we isolate the muscle elements, either imperfectly in experiments or perfectly in thought, they do not exhibit the functions of the whole organ, but only those connected with contractility Such isolated smooth muscle elements from various organs are not uniform as to excitability, mediation, inhibition, and conductivity The contractility they have in common, but they share this with striated muscle and even with other vertebrate tissue elements, such as chromatophores, ciliary epithelium, etc. Thus vertebrate smooth muscle must be defined as Cells specialized for contraction only with their protoplasmatic constituents homogeneous in the longitudinal direction

In vertebrates, only in exceptional instances do these smooth muscle cells unite, without important participation of other tissues, to form something like an anatomical muscle e.g , the pilomotors in mammals, the retractor penis in some species But in invertebrates, the formation of anatomical muscles from smooth muscle cells is rather common, and since a large part of the experimental work on smooth muscle has been done on invertebrate material, the above outlined difference between vertebrate smooth and striated muscles has often been neglected.

Due to the fact that we have a relatively well established visualization of the physiological properties of striated muscle, it has become customary to express the function of smooth muscles in terms derived from the function of striated

muscles, although often these terms might have little meaning for smooth muscles. But such terms, if used discriminately, help to shorten description and discussion. In a review it is impossible to discuss the properties of the smooth muscle elements separately for each organ and to point out each time the differences between various species. An attempt will be made to show that, on the one hand, smooth muscle tissues of various origins have in common certain properties directly connected with contractility while, on the other hand, there exist important organ and species differences of those properties connected with excitation and conduction.

DOES THERE EXIST AN INTERMEDIATE MUSCLE TYPE? Only during embryological development and in tissue cultures do difficulties arise in classifying muscle cells as smooth or striated. In those exceptional instances in which striated muscle fibers participate with other tissues in forming organs, they have the physiological and the pharmacological properties of true skeletal muscle fibers, i.e., the striated fibers of mammalian esophagi (88, 134), or the striated fibers of the iris of Sauropsidae (33, 132, 164). The latter example is of special interest, since these striated fibers are of ectodermal origin (145), and the dilator at least is smooth in some species of birds (93). A similar variability in the composition of the esophagus musculature is found not only from class to class of the vertebrates (178), but also from species to species in mammals (216) and even between related sub-species (239). But never the least histological or physiological evidence was found for the existence of an intermediate type of muscle cells. In the palatal membrane of many fishes (37, 163), and in some species (i.e., tench) throughout the whole gut (95, 159) striated muscle fibers are found scattered more or less numerously through the smooth muscle coats. Physiologically and pharmacologically, the striated behave like true skeletal muscle, and the smooth ones like gut muscle in general.

STRUCTURE. Despite the diversity of opinion about the nature of the surface of the smooth muscle cells (membrane or only border sheets of hardened sarcoplasm), the histologists agree (107) that a typical membrane comparable to the sarcolemma of the striated muscle does not exist and that in many organs containing smooth muscles, the fibrils run from cell to cell and also form a loose fibrillar net by anastomoses with neighboring cells, thus giving rise to a syncytium-like formation. Roskin (203) points out that in most smooth muscle tissues the smooth muscle cell does not represent a unit and that on the other hand the network of fibrils has not all of the characteristics of a syncytium. He proposes the name "myon" for a single strand of smooth fibrils which can form "myons of higher order" by protoplasmatic anastomoses. There is no doubt that the more superficial fibrils (boundary fibrils) in a cell are coarser than the more central ones. There is a large variation in the number and thickness of the fibrils from one smooth muscle organ to another.

For smooth muscles from the cat intestine two different histological states of contraction have been described (203). In one, the fibrils are straight, while in the other state the fibrils are curled regularly like corkscrews. However, in experiments with the retractor penis and intestinal loops of dogs, it could be

demonstrated that curled fibrils are never seen if the muscles have been fixed histologically after slow contraction against a heavy load. But corkscrew fibrils are found in the inner part of muscles if they shortened quickly before or at fixation (88) These spiral fibrils apparently belong to relaxed myons and are crumpled together by the contraction of adjacent myons

The submicroscopical structure of vertebrate smooth muscles (retractor penis, gut) as investigated by birefringence measurements revealed marked conformity with that of the A disc of the striated muscle (88) Besides a micellar pattern, there exists a crystalline structure of the micellae themselves As in striated muscle, the submicroscopical pattern is disarranged during isotonic contraction, but little change occurs during isometric contraction The small differences observed indicate that the molecular configuration of the two myosins is not identical but only similar, as is indicated also by the similar but not identical flow birefringence and solubility properties for myosins extracted from smooth and striated vertebrate muscles (160)

Efferent innervation The majority, if not all, of the organs containing smooth muscle elements receive efferent nerve fibers from both divisions of the autonomic nervous system However, this is not valid evidence for assuming that all myons are under direct influence of both orthosympathetic and parasympathetic impulses This can only be proved experimentally, but various investigators have often reported controversial results and conclusions for the same organ of identical species As the most striking example the dilator iridis may be mentioned, the existence of which is doubted by Langworthy and Ortega (138) The dilator action still present after elimination of any sphincter action they explain plausibly by the diminished blood flow through the iris brought about by arterial constriction caused by the stimulation of the orthosympathetic nerve fibers entering the iris.

For most of the organs, the main difficulty in determining the exact innervation of the muscular elements is due to the well known fact that, as a rule, at least the pre-postganglionic synapses for the parasympathetic division are situated in close proximity to the muscle elements. Furthermore, only for a limited number of peripheral ganglia, in which these synapses are located, has their purely parasympathetic nature been established beyond doubt. Auerbach's plexus of the gut, the largest and probably also biologically the most important peripheral ganglion, is claimed by most textbooks as purely parasympathetic. However, investigators who claim that only parasympathetic synapses are present in this plexus have to admit that orthosympathetic fibers run through the aggregation of nerve cells In their opinion these fibers do not participate in synapse formation (e.g., 125) Stöhr (223) takes the stand that histologically the nature of the myenteric plexi cannot be claimed with certainty, while Patzelt (176) states definitely that both autonomic divisions participate in the formation of synapses, but that the majority of the entering fibers are parasympathetic.

When nerve fibers, either originating from nerve cells of the ganglia or having passed without interruption throughout the latter, approach the muscle cells, a classification as orthosympathetic or parasympathetic ones is practically impos-

sible, since both are now non-myelinated fibers of extremely small diameter Tiegs (228, 229) was probably the first who realized that in a number of organs the finest nerve fibers form a true network enveloping the smooth muscle cells and finally entering them He emphasized the physiological importance of this fact Such a terminal network has been now well confirmed for the urinary bladder (207, 222), ureter (207), intestinal tract (31, 223, 224, 225), bronchi (119), arrector spinorum of hedgehog (32), iris and ciliaris muscle (33, 35), uterus (208), and blood vessels (32, 34, 223) Stöhr (226) claims that orthosympathetic and parasympathetic nerves unite in the "terminal reticulum" and Boeke (36) also assumes sympathetic and parasympathetic participation in the "terminal syncytium" They use a different terminology for this terminal nerve network, since Boeke regards the interstitial cells of Cajal as an integral part of the network But since he does not assume any ganglion cell-like activity of the interstitial cells, this difference in opinion is of no interest for the physiologist After section of the macroscopical nerves, the nerve reticulum shows only minor degenerative changes The absence of full degeneration might be due to the presence of the interstitial cells Section of the nerves of only one autonomic division still produces less degeneration Further confirmations of the existence of such a terminal nerve reticulum were reported by Lawrentjew (144), Reiser (186), Leeuwe (143), and Steffanelli (219), while Clark (66) and Nonidez (170, 171) deny its existence and find the nerves ending on the surface of the muscle cells and not entering them According to Nonidez the so-called terminal reticulum is in reality a network of argyrophil minute connective tissue fibers, also found in liver, thyroid and other glands However, this network in glands has been interpreted by Boeke and others as a terminal nerve reticulum Why these fine connective tissue fibers and not the coarser ones should be argyrophil has not yet been explained by the opponents of the nerve reticulum view, on the other hand it is well known that all neurofibrils are argyrophil It is understandable that the acceptance of the existence of a true nerve reticulum is difficult for an adherent of the classical anti-continuity neurone theory That vertebrate axons can fuse to form a nerve net has been demonstrated in tissue cultures (e g , 14, 15)

This intensive controversy between the histologists has brought out a further fact important for physiological discussion Contrary to the old belief that nerve endings exist only on a small minority of the individual smooth muscle cells, all recent investigators agree that as a rule nerve endings are found at least for a majority of the muscle cells

Such a reticulum formation by the efferent fibers explains why in no type of physiological experiment has a true systematization of smooth muscle organs in "motor units" been found The pilomotors react more or less as a single unit after nerve stimulation and the all-or-none rule is demonstrable to a certain extent (191) This can be explained, despite the nerve net which probably conducts with a decrement as in invertebrates, by the minute spatial dimensions of one single pilomotor and by the probable, but unproved, fact that the nerve net of each pilomotor receives only one postganglionic efferent nerve fiber It is re-

grettable that no physiologist has used for experimental purposes the much larger arrectores spinorum of the hedgehog The nictitating membrane, which in certain physiological respects behaves similarly to the pilomotors, is not divided into motor units, and the all-or none rule does not apply to it (64, 191, 193, 201) Klopp (131) found in experiments with partial denervation of the membrane that localized fractions of the muscles become hypersensitive to epinephrine. This localized influence, for which no sharp demarcation has been demonstrated, does not mean necessarily an organization in units, since in a nerve net conducting with a decrement, efferent fibers connecting with the net will have a more marked influence upon the function of the net around the point of connection than upon the more distant parts This might explain, too why stimulation with microelectrodes of the minute nerves leading to small arteries of frogs exerts a spatially limited effect (96) For the dilator iris of the cat, experiments with stimulation of all or only a part of the efferent nerve fibers revealed the absence of true motor units and no evidence even of any spatial preferences (149)

Afferent innervation How far the terminal nerve net is able to carry afferent or antidromic impulses for axon reflexes is a question which can be answered at present only by conjectures For all organs containing smooth muscles, there exist true afferent myelinated fibers, which have their nerve cells in the dorsal root ganglia "Spray like" or "spindle like" sensory endings are found scattered between the myons of the urinary bladder (122, 137), of the sphincter iridis (138) and of the stomach (139) and are probably present in other smooth muscles too Action currents of the afferent nerve fibers in response to stretch of the muscles have been demonstrated for the urinary bladder of the frog (221) and for the cat bladder and urethra (82)

Ganglion cell-free smooth muscle preparations Unfortunately for the physiological analysis of smooth muscle activity, the synapses in the efferent nerve pathways are not all aggregated in the anatomical ganglia, so that a variable number of true ganglionic synapses are found scattered between the myons Thus in any analysis of the motor activity of smooth muscle organs, one has not only to ask how far is the observed property a function only of the myons proper or of the terminal nerve reticulum, but one has to consider also the ganglion cells Absolutely free of nerve cells are the pilomotors (32, 68), the iris muscles (33, 35), the retractor penis (243, 244) and probably the nictitating membrane (195) There is no doubt that in many species, large parts of the uterus do not contain nerve cells (72, 104, 187, 208) Cajal's interstitial cells found abundantly in the uterus and gut are no longer regarded as primitive ganglion cells by most histologists, although a few still maintain the old view (146) The muscularis of arteries is free from nerve cells since the latter are all found in the adventitia (144, 223) In the muscle layers of the gut, a few true ganglion cells are scattered between the myons But their number is so small that the preparation of ganglion free strips is possible for physiological experiments, although a later histological examination is indispensable (98, 236, 237) Such strips show nearly as much automatism as strips containing Auerbach's plexus and their reaction to acetylcholine and epinephrine is not altered qualitatively, but only quantitatively Whether this

small diminution in activity is due to the absence of ganglion cells or due to unavoidable damage during the cumbersome isolation of the strips, is not yet known

Smooth muscle completely devoid of any nervous tissue is found in the amnion of the chick embryo (83) and in tissue cultures of embryonic gut (204, 227) Both these muscles contract under the influence of acetylcholine (prevented by atropine at least for the amnion muscle) and are inhibited by epinephrine Euler (80) demonstrated in perfusion experiments on the nerve-free vessels of the human placenta that epinephrine always produces constriction, while acetylcholine influence varies from nil to dilatation or even contraction

All this evidence indicates that the two chemical mediators can act on the muscle substrate directly, and that neither terminal nerve reticulum nor nerve cells are essential for these reactions However, the task of analyzing, for these two mediators, their exact sites of action and their relative importance for smooth muscle organs not devoid of nerve cells, is almost impossible The stimulating action of acetylcholine upon all ganglion cells is well known (56, 64), and that epinephrine can affect the acetylcholine mechanism has been demonstrated recently (62, 220, 230) About the effect of these two drugs upon the terminal nerve reticulum little is known The well known increase in acetylcholine or epinephrine sensitivity after degeneration of the nerves tells us nothing with certainty about the site of the increased sensibility, since the terminal nerve net undergoes only minor degeneration

That, even in the absence of nerve cells, the mechanism of the mediator action can be a very complicated one has been shown for the pilomotors Intracutaneous acetylcholine injections around these adrenergic muscles produce contraction, such a contraction is still produced after section of the orthosympathetic nerves. However, the acetylcholine contraction cannot be elicited after the nerves are degenerated Coon and Rothman (68) explain these findings by assuming axon reflexes, the receptor end possessing several properties of autonomic ganglia

Acetylcholine and epinephrine antagonism The smooth muscle effectors have often been classified according to their excitatory mediator into adrenergic and cholinergic ones (56, 192) The apparent antagonistic effect of parasympathetic and orthosympathetic nerve stimulation for certain organs has been explained by the presence of two antagonistically arranged muscle sets, one cholinergic, the other adrenergic For the purposes of this paper a discussion of the differences between sympathin E and sympathin I and of their chemical nature (64, 97, 103, 192) is irrelevant, and, therefore, the expression "epinephrine" is used to designate any adrenergic mediator Besides the existence of two types of smooth muscles activated by two different mediators, it has been demonstrated that certain smooth muscles are not only excited by one of the mediators but also distinctly inhibited by the other The best known examples are the gut muscles, for which a reciprocal quantitative antagonism between the excitation by acetylcholine and the inhibition by epinephrine was revealed by Bernheim (28) A similar relation between the effects of the excitatory adrenergic nerve endings and the inhibitory cholinergic ones was found for the retractor penis (173) A large

number of the systemic arteries are contracted adrenergically and relaxed cholinergically (i e , 156) while on the coronary arteries (i e , 101) and on the thoracic duct (2) the two mediator actions are just reversed On the pulmonary arteries, the two mediators act differently but not as true antagonists (79, 91) The sphincter iridis, at least in some species is under such an antagonistic control of the two mediators (21, 105, 120, 180) For other contractile vertebrate cells, the chromatophores of fishes, the antagonistic influence of mediators or neurohormones is well established (99, 175)

The question of whether those muscles with a well established antagonistic innervation receive nerve fibers from both divisions of the autonomic nervous system (i e , retractor penis, 173) or only from one division (i e 61, 63 153, 156) is of minor interest from a theoretical point of view but of practical importance in the analysis of the results of stimulation of a single anatomical nerve

A further complication in the analysis of the innervation of smooth muscles is the fact that in some organs some of the myons are adrenergic and others cholinergic in the excitation of contraction although no true mechanical antagonism exists between the differently innervated myons The external layer of the pigeon esophagus is adrenergic and the internal layer cholinergic (111) The stomach muscles of at least some species are partly adrenergic and partly cholinergic (57, 169) In the nictitating membrane the majority of the muscle cells are adrenergic, but a minority cholinergic (4, 10)

Seasonal, hormonal and vitamin influences The complexity of the innervation mechanism of smooth muscles, which makes any valid general explanation of excitation and conduction phenomena impossible, is increased by the fact that, even for a given muscle, the mediator sensitivity is fluctuating The seasonal influence is probably most marked for the smooth muscles of the frog lung (69, 242) *The hormonal influence on the activity of the uterus* is best known The effect of the various sex hormones is different from species to species, but can produce in some species an apparent reversal of the effect of nerve stimulation (30, 58, 187) The motor activity of the vesicula seminalis and of the ductus deferens is also affected by sex hormones (157, 158 235) Urinary bladder (136 137, 240) and ureter (126, 231, 232) in the females are affected in a comparable manner but less so Even on the gall bladder (217) and the intestinal tract (84, 206), the influence of the sex hormones is demonstrable The relation between thyroid activity and sensitivity of the adrenergic mechanism has met with some attention (i e., 8, 121, 142 ) Several of the vitamins can also alter the effective innervation and activity of smooth muscles Thiamine enhances certain cholinergic mechanisms (1, 53) nicotinic acid influences slightly uterine and intestinal activity (147), and ascorbic acid under certain conditions increases adrenergic as well as cholinergic activity (106, 133)

Action of drugs A discussion of the various drugs affecting smooth muscles is beyond the scope of this paper, and a number of fairly recent reviews of this subject are available (56, 123, 188, 192) However, a few drug actions must be discussed here on account of their implications for the physiology of smooth muscles in general Histamine, in more or less physiological doses, contracts

nearly all smooth muscles, the adrenergic and the cholinergic ones This has been demonstrated for the intestine (25, 26, 27, 28), bronchi (85), nictitating membrane (190), gall bladder (52, 124, 127), uterus (233, 240), iris (108), veins (90, 155), coronary arteries (13), and lung arteries (85, 172) Systemic arteries are contracted too in perfusion experiments with histamine in saline, but the presence of a few red cells and of small amounts of epinephrine changes the contraction effect to dilatation, as shown by Dale and Richards in their classical investigation (71) A similar protective mechanism may be the reason why histamine contraction has not yet been demonstrated for those smooth muscles not mentioned above (i e pilomotors) Nearly all investigators agree that the histamine effect is still present after paralysis with atropine or ergotoxin This can be accepted as evidence that histamine acts neither on the theoretical "receptors for acetylcholine" nor on the "receptors for epinephrine," but on some link closer to the contractile mechanism It is of interest in this connection that Ambache and Barsoum (7) reported a release of histamine for gut and bladder of guinea pigs and rabbits from muscles contracted by acetylcholine, KCl, or pituitary extracts This histamine liberation is absent after corresponding treatment of muscles paralyzed by atropine More observations of this type, if possible also on adrenergic muscles, are needed before far-reaching conclusions can be drawn

Although not enough data are available to make a general statement, there are indications that angiotonin contracts not only smooth muscles of arteries, but more or less all smooth muscles, and that it acts also on a link closer to the contractile mechanism than the receptors for the mediators Angiotonin action on the systemic arteries is not prevented, as epinephrine action is, by F 933 or ethyl yohimbine (5, 247) Ergotoxine, however, prevents epinephrine and angiotonin action (241) Strong angiotonin contractions have been demonstrated on the gut (67, 112, 174), uterus, bladder and nictitating membrane (5, 152) Distinct, but not quite such strong angiotonin effects were observed for ureter, gall bladder, ductus deferens and bronchi muscles by Ludueña (152)

The well known paralyzing effect of atropine upon cholinergic myons has one feature which must be discussed here For some muscles, atropine prevents only the effect of externally or intravenously applied acetylcholine but diminishes very little or not at all the effect of parasympathetic nerve stimulation (70, 122, 123, 169) The current explanation that atropine prevents diffusion of acetylcholine through cell membranes but does not prevent intracellularly liberated acetylcholine from reaching the receptors, assumes that in those muscles in which atropine blocks nerve impulses, nerve endings exist only on the surface of the muscle cells, while in those muscles in which no nerve block occurs, all nerve endings are intracellular For those muscles with partial nerve block produced by atropine, it is believed that some of the nerve endings are intracellular and some extracellular Corresponding observations and conclusions have been made for the effect of ergotoxine upon excitatory adrenergic muscles, where also apparently diffusion of epinephrine into the cells is prevented, but not mediation from an intracellular nerve ending (i e, 64, 129, 190, 192) The explanation that only a

limited number of muscle cells is innervated at all and that normally the impulse is transmitted from the directly innervated cells by diffusion to the non innervated cells can hardly be maintained in view of the present histological evidence mentioned before Unfortunately, the question of histological and physiological innervation of a smooth muscle organ has never been investigated by a combined team of histologist and physiologist, both well acquainted with the methodological difficulties of the problem

Some doubt about the value of atropine and ergotoxine as tools for exploring the excitatory cholinergic or adrenergic nature of smooth muscle innervation has been expressed by Mellanby and Pratt (161, 162), who believe that at least some of the paralyzing effect is not due to a specific blocking but to a general "immobility" of the muscle substance proper caused by these drugs Also some experience makes it doubtful that the only effect of ergotoxine is a blocking of excitatory adrenergic mediation (i e , 4, 169, 151, 205)

SENSITIZATION OF DENERVATED SMOOTH MUSCLE TO ITS MEDIATOR After section of the postganglionic nerves, smooth muscles do not degenerate but show always an enormously increased sensitivity for their excitatory and their inhibitory mediators (70, 110, 150, 210) After regeneration of the nerves, the mediator sensitivity declines again to normal values Section of the preganglionic nerves produces much less sensitization (212) or no sensitization at all (248) according to which muscle is used in the experiments Increased permeability of the muscle cell membranes is generally accepted as the mechanism of this sensitization for the specific mediators (64) This also explains why simultaneously the sensitivity is increased for drugs which act on links, which are closer to the contractile mechanism than are the receptors for the mediators (e g histamine, 190)

ELECTRICAL EXCITABILITY One of the outstanding features of the postganglionic-denervated nictitating membrane is the apparent absence of electrical excitability (34, 75, 167), this excitability can be restored, to a certain extent, by treating with the drug F 933 Unfortunately only few smooth muscles allow complete postganglionic denervation The rectractor penis, probably very suitable for this type of experiment, has apparently not been used Pilomotors are completely unexcitable electrically after denervation (196) Chronic denervation is at the moment the only experimental condition in which the electrical stimulus might act on the muscle cells alone, if the slight degeneration of the nerve reticulum excludes stimulation of it. The intimate relation between terminal nerve reticulum and muscle cells excludes the use of micro-electrodes, and the blocking by atropine or ergotoxine does not affect intracellular nerve endings. The nerve-free muscle cells of the chick amnion are electrically excitable, but rather strong currents are apparently needed (179)

CHRONAXIE. Nearly all chronaxie measurements have been made on organs or on strips of organs containing smooth muscles besides various nervous tissues Therefore it is more or less guess work if one attributes the measured chronaxie either to the muscle cells proper or to the nervous elements The chronaxie values reported are all rather long, at least 15 times as long as for skeletal muscles of the same species (37, 43, 81, 188) and much longer than the chronaxie values

for the slowest vertebrate nerve fiber yet measured However, the diameter of the nerve fibers forming the terminal reticulum is also much smaller than that of the smallest nerve fiber subjected up to now to direct chronaxie measurement The strength-capacity curves determined for the nictitating membrane (213) revealed typical breaks into an $\alpha$ and a $\gamma$ segment Again, it is more or less arbitrary whether one ascribes the higher $\alpha$ excitability to nerves and the $\gamma$ excitability to the muscles proper, or whether one assumes that the higher excitability is that of the somewhat larger nerves leading to the nerve net, and the lower excitability is that of the finest fibers forming the terminal reticulum No break in strength-duration curves was observed for the ureter of rabbits and for the uterus of rabbits, guinea pigs, and cats by Bozler (43) He regards the strips of these organs as true muscle syncytia like the heart, since they follow the all-or-none rule and since conduction is not much disturbed by zigzag cuts However, all these observed properties can be ascribed as well to the terminal nerve reticulum (228, 229)

For some smooth muscles, the chronaxie varies with the season (94) In some species the uterus is not electrically excitable in anestrus but excitable in estrus, and it can be demonstrated that chronaxie shortens with increased influence of estrogens (43, 130) It would be of value if, on the same muscles, the changes brought about by seasonal and hormonal influences in chronaxie and in mediator sensitivity could be studied simultaneously

Electrical stimulation with variation in frequency and slope of the current shocks has not yet been reported as stimulating selectively either the excitatory or the inhibitory mechanism of isolated vertebrate smooth muscles responding antagonistically to the two mediators However, on single nerves leading to such structures selective indirect stimulation of the excitatory and the inhibitory mechanism has been possible (156, 209, 238) According to Lapicque (140), all autonomic nerves are "iterative," which means that only a summation of arriving volleys can elicit the reaction of the effectors At least for the excitatory nerves of the nictitating membrane, of bladder and of pilomotors, it could be demonstrated that a single nerve volley can produce contraction (55, 76, 199) The same has been found for the excitatory vagal fibers leading to esophagus and stomach of the cat (154) For no inhibitory nerve of vertebrate smooth muscle has such a single-volley effective yet been demonstrated, but the inhibitory vagal heart mechanism is not "iterative" (86, 100)

CONTRACTION TIME AND LATENT PERIOD One of the few general physiological characteristics of vertebrate smooth muscles is the slowness of contraction Smooth muscle contractions elicited by single indirect or direct stimulation last at least 15 times as long as a twitch of any striated muscle of the same species (20, 135, 154, 213) The ratio between contraction time proper and relaxation time is always lower than in striated muscles Thus relaxation is as a rule a very slow process, a fact which must be kept in mind for any discussion of the tonus problem For those smooth muscles with inhibitory innervation or inhibitory mediator mechanism, relaxation can be speeded up and might become quicker than the contraction phase (41, 42)

Due to the intimate anatomical relation between nervous tissue and muscle cells and due to the slowness of the mechanical process, any determination of the mechanical latent period for smooth muscle organs has limited value only. This latent period, which lasts at least 0.15 sec. but often reaches much longer values (135, 154, 199), varies not only from organ to organ and species to species, but also for different parts of the same organ (i.e., intestine, 6). If the postganglionic nerves of the nictitating membrane are stimulated by single shocks, the latent period declines from 0.4 to 0.15 sec. with increasing strength of the stimuli (76).

REFRACTORY PERIOD. In experiments with mechanical recording the refractory period can be determined only as the minimal interval between two successive stimulations still producing summation. For the stomach of the cat, McSwiney and Robinson (154) report a refractory period of 0.8 sec., while for the nictitating membrane, Brown (55) reports a refractory period of 2 msec., which was more or less confirmed by Rosenblueth and Acheson (194), while according to Eccles and Magladery (76), as judged by electrical evidence, some delay in summation occurs for intervals shorter than 55 msec. This large difference between refractory period of the stomach and that of the nictitating membrane might find an explanation in the observation of Umrath (234) that two different refractory periods can be demonstrated on the frog rectum. The "autogenous" refractory period, that is, that of the muscle cells themselves, is very short, less than 10 msec., and is lengthened after two excitations and by fatigue or narcosis. The "induced" refractory period, which is forced upon the colon by Auerbach's plexus, acting as "pacemaker", lasts about 20 sec. and is shortened after two excitations and by fatigue or narcosis. It must be pointed out, however, that ganglion-free muscle organs also can have rather long refractory periods (e.g., ureter, uterus, 43). Has the induced refractory period been measured in those muscles, and is the terminal nerve reticulum (or the interstitial cells) responsible for the induced refractory period? Since in such muscles pacemaker functions have been demonstrated (50), a positive answer to these two questions might be permissible.

CONDUCTION. Monnier (166) demonstrated for isolated arteries that contractility and conduction are, at least for these muscle organs, not identical processes since conduction can occur without contraction of parts of the artery through which conduction has taken place. In the retractor penis, local electrical stimulation produces only more or less localized contraction (243). On the same muscle, mechanical stimulation applied strictly locally without stretch of other muscle parts, also produces only local contraction (88). On the other hand, there is no doubt that some smooth muscle organs not containing ganglia show conduction. For oviduct, ureter and uterus (in estrus) conduction rates between 2 to 60 mm/sec have been found (43, 49, 116, 168). It is possible, too, that in some organs under certain conditions, e.g., uterus, excitation by nerve stimulation is localized and non-conducted as is chemical stimulation, while direct electrical stimulation elicits a conducted process corresponding to that occurring during spontaneous movements (193).

ACTION POTENTIALS Not only because of the diversity in anatomical arrangement and in function of the various smooth muscle organs, but also because of certain methodical difficulties in recording electrical potentials and their changes, the findings and the interpretations of various investigators are rather controversial The majority of the investigations published before 1935 were made without amplification, with string galvanometers, the strings were very loose to secure the needed high sensitivity With such a method, potential changes of slow frequencies can be recorded more or less correctly, but not those of relatively high frequencies The use of amplifiers in connection with various recording instruments permits the recording of high frequency changes of minute potentials, but does not allow the accurate recording of slow potential changes Furthermore, many investigators using amplifier technic, did not record simultaneously the potential changes and mechanogram Thus, any time relation claimed between potential changes and contraction becomes more or less arbitrary

As mentioned above, one of the relatively simply organized smooth muscle organs is the *pilomotors* However, even their potential changes, occurring after single shock stimulation of their nerves, are rather complicated and vary a good deal (135, 198, 200, 201, 202) Of the seven different potential waves observed, only the four main ones will be mentioned here The first two are rapid potential changes in quick succession Potential II slightly precedes or is simultaneous with the onset of contraction Potential IV is a slow wave which follows closely the course of contraction, but is not an artefact due to contact changes on the electrodes or due to changes in shape of the tissue between the electrodes Potentials III are some medium quick waves, not always present, superimposed upon the slow basic potential IV The presence of such superimposed waves upon the contraction potential is not surprising since the mechanogram of the pilomotors often shows some superimposed oscillations (20) Potentials I and II increase upon repetitive stimulation, while I, III and IV, but not II, disappear after ergotoxine paralysis Epinephrine in small doses, which do not contract the non-sensitized pilomotors, decreases potentials I and II If the pilomotors are sensitized by cocaine, the same dose of epinephrine produces contraction, and the depressing action of epinephrine upon potentials I and II elicited by nerve stimulation is enhanced

The *nictitating membrane* gives rather similar records (135, 198, 200, 201, 202) The main difference is that the potential change occurring parallel with the contraction can be subdivided much more clearly and constantly into the basic potential IV and the superimposed quicker potential III, which is of varying character monophasic, biphasic, polyphasic, or distinctly repetitive during the whole contraction With repetitive stimulation of the nerves, potential I usually shows a decline, while II becomes more marked Cannon and Rosenblueth (192), assuming nerve endings on only a limited number of muscle cells, tentatively identified the potential I with depolarization of the directly innervated key muscle cells, potential II with depolarization of adjacent non-innervated muscle cells, and potential IV with depolarization of other interfaces and with contraction Eccles and Magladery (76, 77) more or less confirmed all these findings, but ob-

served a further small wave N preceding potential I N is even present when the nerve stimulus is too weak to produce contraction and potentials I and II. The interpretations of Eccles and Magladery, however, are quite different, since they believe in the existence of a fairly long refractory period of the muscle cells and in the conduction of a wave of negativity, propagated distally along the membrane at a rate of 50 to 80 mm /sec., which they regard as the expression of muscular conduction. Rosenblueth (193) emphasizes rightly that the electrical potentials of smooth muscles are too complicated and not sufficiently understood to draw conclusions concerning the properties of smooth muscles contradictory to other evidence The anatomical arrangement of the muscle cells in the nictitating membrane renders muscular conduction rather unlikely (3) Reinvestigation of the potentials with special search for any indication of conducted negativity waves or of refractoriness of the muscles confirmed their absence (202) During epinephrine contractions, the potentials observed resemble very much potential III and IV after nerve stimulation (77, 167, 198, 199) Nerve stimulation, while the membrane is under the influence of epinephrine, produces electrograms in which the potentials I and II are diminished or even completely absent (64), although the muscle still responds with further contraction

For the *urinary bladder* of the cat after stimulation of the pelvic nerves, potentials rather similar to those for pilomotors and nictitating membrane were recorded (135, 199), and are similarly interpreted as of local origin and not conducted.

It is regrettable that the potential changes of the *retractor penis* apparently have never been studied with modern technic, since this muscle is very suitable for studying the problem of conduction The string galvanometer record of Brücke and Oinuma (59) shows indications of small spikes and large slow wave components, the latter probably comparable to potential III

The ganglion cell-free *ureter* of the pig shows regular contraction waves starting from the renal end, which are accompanied in string galvanometer records by mono- or di phasic potentials according to the placement of the electrodes (115) Each wave lasts for several seconds, and their frequency varies between 2 and 8/min. The amplitude and frequency increases from renal to vesical end. Amplitude of the potential and strength of contraction show no strict correlation Bozler (44, 49, 50, 51) reinvestigated the ureter of various species with the amplifier technic and found, with a monophasic lead for the guinea pig, discharges up to 40/sec during a peristaltic wave However, in the rabbit and especially in the rat, the action potentials are rather slow negativity changes (lasting up to 3.5 sec. in the rat) Bozler is inclined to explain these long lasting action potentials by a burst of impulses fused into a continuous state. At the pacemaker or the renal ureter end, Bozler observed small local negative potentials developing before the outburst of conducted impulses Isometric, but not simultaneous, recording of the mechanogram revealed a small tonus increase preceding the start of a contraction at the site of the pacemaker According to Bozler, both the tonus increase and the small negativity are expressions of a local depolarization.

During the spontaneous contractions of the *ductus deferens* of cats, waves of negativity, more or less parallel in time with contraction can be detected with the string galvanometer (9) In corresponding experiments slow mono- or di-phasic potentials, lasting 3 to 9 sec each, have been found in rabbits (117)

The contractions of the *vagina* of rabbits give negative potential waves of 5 to 12 sec duration coinciding with contraction (38, 39) Similar waves lasting about 9 sec and travelling at a rate of 1 7 to 3 3 mm /sec have been observed on the isolated *oviduct* of pigs by Hasama (116) He believes that the occasionally recorded superimposed waves of higher frequency (2/sec ) are due to "interference" of the potentials of parallel muscle fibers

The electrical activity of the *uterus* is complicated by the already discussed influence of the sex hormones upon excitability and conduction With the string galvanometer, slow negative potential waves were observed by Hasama (113, 114) on the virgin rabbit uterus with a frequency of 3 to 7/min , more or less synchronized with contraction waves However, there was no constant parallelism between amplitude or frequency and the strength of contraction In the isolated uterus, oxygen lack depressed the potentials much less than the contractions As a rule, sympathetic drugs increased amplitude and frequency of the potentials, while parasympathetic drugs only increased the frequency For the cat, essentially similar potentials were recorded by Bacq and Monnier (9) Epinephrine increased the potential waves and the contractions of the gravid uterus, but inhibited both phenomena in the nongravid uterus In the latter, potentials and contractions were increased by acetylcholine

Although Greene reported in 1928 in a short note (102) that, besides the slow waves of the uterus, there also occur rapid repetitive deflections, our knowledge of the electric activity of the uterus was really clarified only 10 years later by the investigations of Bozler The latter not only demonstrated for the guinea pig, rabbit and cat that conduction and electric discharges are dependent on a certain minimal level of estrogens (43, 48), but he was also able to record rather quick potential waves by the use of amplifiers (44, 46, 47, 51) Unfortunately, the contractions were not recorded simultaneously These high frequency waves can be obtained from the conducting uterus either monophasicly or diphasically The conduction rate is slow for the guinea pig (1 to 3 mm /sec ), but higher in the two other species (up to 60 mm /sec ) The single waves have varying shape but are all rather short, and bursts of impulses up to 40/sec were observed Sympathetic nerve stimulation or epinephrine lowers or abolishes the potential waves and their conductivity in the non-pregnant uterus of the cat, but this inhibitory effect is often preceded by a short excitatory state Bozler assumes that this diphasic response is due to a diminution in excitability which leads to a block of muscular conduction, but that simultaneously new local impulses are set up The excitatory action will be suppressed as soon as the excitability level has dropped sufficiently to block conduction Morison (168) confirms these results in the main and emphasizes the local action of epinephrine and of nerve stimulation During prolonged epinephrine contractions, not only the slow waves are present but also the quick, spike-like potentials The slowing action of epine-

phrine in the non pregnant cat uterus is only present if the drug is applied to the pacemaker at the uterus horn. Balassa (11), using the string galvanometer and recording simultaneously the contractions, observed for rabbits in anestrus that slow potential waves occur with the weak contractions, and that the more spike-like potentials are only present during the more active contractions during estrus or late pregnancy For the cat, Balassa and Gurd (12) also report slow waves in anestrus, but for estrus and late pregnancy, high frequency potentials in short bursts occurring only at the start of spontaneous and of drug contractions They assume with Bozler that only when the estrogenic hormones predominate is conduction from muscle cell to muscle cell possible For the human, and also for the dog, high frequency potentials during spontaneous and pituitrin contraction of the uterus have been observed (128)

Puestow (181, 182), as well as Berkson (22, 23, 24), reported electromyographic studies of the *intestine* in situ or after isolation of the rabbit, dog, cat, guinea pig and rat. With the string galvomometer without amplifier, monophasic or bi phasic records are obtainable, they are rather variable even for the same species However, in all cases a slow negative wave occurs parallel with the mechanogram during a peristaltic wave. Rapid spike-like potentials of various shape are superimposed on the basic potential wave during the contraction proper Such rapid potentials are present, too, during long lasting contractions produced by direct repetitive electrical stimulation. However, occasionally complete spontaneous dissociation of the electrical and mechanical activity occurs in apparently normal loops. Curare increases the strength of spontaneous contractions without altering the electrogram Epinephrine paralyses the loops without abolishing the electrical activity completely Eserine increases the motor activity and the number and frequency of the spike potentials Nicotine applied to an isolated loop produces at first strong contractions with quick electrical oscillations, but after return to normal motor activity, not only the quick but also the slow electrical waves are absent Nicotine applied to a loop in situ has little effect upon the spontaneous contractions, but abolishes all the electrical signs if the circulation of the loop has been diminished by arterial compression. Restoration of circulation will restore normal electrical activity with little change in the mechanogram Puestow and Berkson conclude that the electrical potential changes originate within the intrinsic nervous plexus The slow potential waves were reported, too, for the rabbit stomach (118) Paulian and Felice (177) claim that in the cat intestine the onset of the slow negativity wave precedes spontaneous and also acetylcholine contractions, but no tracings are given in their paper

Bozler (44, 45, 46, 49, 50, 51) recorded with amplifier, but without simultaneous mechanogram, the potential from isolated intestinal strips and from loops in situ for the dog, rabbit, cat, guinea pig and rat. No slow potential waves were observed in his experiments, but occasionally long lasting after potentials Although the electrograms varied not only from species to species, but also from one type of contraction to another (pendulum movement, peristalsis, etc.) there was one common characteristic, namely, the presence of bursts of repetitive dis-

charges of spike-like potentials The frequency ranged from 1/sec for the cat stomach to 10/sec for a peristaltic rush of the small intestine of the rabbit These quick potentials can be recorded either as monophasic or as biphasic ones and are conducted waves of negativity Bozler believes the potentials are too large to be produced by the relatively small amount of nervous elements present, and assumes muscular origin and muscular conduction through protoplasmic bridges from muscle cell to muscle cell Epinephrine, in doses not high enough to stop the intestinal movements completely, does not diminish the frequency of spontaneous movements, but only their strength, and the number of electrical discharges found is accordingly diminished Bozler explains this by a lowering of the average level of excitability by epinephrine As for the pacemaker of the ureter, slow local potential changes are almost continuously present in all regions of the intestine Impulses, often in bursts, are discharged at the crest of such local negativity waves Forster and co-workers (92) were able to demonstrate bursts of initial spikes at the onset of contractions in the human intestine, these are followed by a long-lasting base line shift with superimposed intermediate electrical activity

For the *gall bladder* of rabbit and guinea pig, slow potential changes and quicker superimposed spike potentials have been recorded with the string galvanometer (16, 17, 18, 148) The slow waves occur more or less simultaneously with tonus changes, while the bursts of spike potentials occur during true contractions

The diversity of the electrograms from various smooth muscle organs makes it impossible to propose any unitary theory for the origin and mechanism of these different action current components There is no doubt that for some of the organs, the spike potentials behave rather similarly to those in skeletal muscles, and that for these smooth muscles, tonus could be explained by gradation in number and in frequency of the impulses (48) It does not matter much for this question if the sites of origin of these conducted potentials are nervous elements or the muscle cells themselves But the fact that complete dissociation between electrogram and mechanogram can occur indicates that the mechanism, which finds its expression in the spike potentials, is probably only of regulatory importance The existence of localized potentials occurring at least in some smooth muscle organs is evident The potentials III and IV of pilomotors and the nictitating membrane are not identical with the slow waves in the intestine, uterus and ureter since the latter are conducted However, there is the theoretical possibility that these slow potentials are localized potentials of the individual muscle cells, thrown successively into activity by conduction through the terminal nerve reticulum and thus simulating a conducted wave of negativity It might not be advisable, since the evidence is not strong enough at present, to generalize with Monnier (165) that depolarization of the muscle cells themselves is constantly associated with contraction, and increased polarization with relaxation

Impedance changes Paulian and Felice (177) tried to support the depolarization theory by measurements of the impedance of the isolated cat intestine They claim that impedance decreases with contraction and diminishes with

relaxation However, as pointed out elsewhere (87), the resistance to alternating currents of high frequency of a muscle fiber is not determined, at least theoretically, by the characteristics of the membrane alone but also by the submiscroscopical structure of the muscle fibrils changing with contraction and relaxation. Careful impedance measurements on simple smooth muscle organs would be valuable for increasing our knowledge about the mechanism of smooth muscle activity

ELASTIC AND MECHANICAL PROPERTIES The differences in the length tension curves for striated and smooth muscles, for the resting state as well as for the contracted state, have been regarded for a long time as one of the main distinctions between the physiological characteristics of these two muscle types. But for the last 20 years it has become more and more clearly recognized that all contractile tissues give qualitatively similar curves, the main difference being only in the time factor (189) If resting vertebrate smooth muscle organs are stretched slowly, the tension increases only a little but linearly for a considerable range, and only for extreme length can considerable tension be produced by slow elongation (retractor penis, 243, blood vessels, 65, uterus, 47, 211, urinary bladder, 60) That the isolated striated vertebrate muscle fiber has also over a long range a constant extension modulus is now well recognized (184) According to Winton (245), the relation between tension, length and time for a stretched or released retractor penis can be represented by a mechanical model including a pure elastic resistance, a viscous-elastic system, and a pure viscous system arranged in series There is no doubt that the absolute values to be assigned to these three components differ a great deal from one smooth muscle organ to another (214, 215) Bozler (48) demonstrated for the muscle coats of the arteries and for the uterus that the rate of extention under a load follows the same time course as the lengthening after a contraction He concludes that relaxation, if not speeded up by an inhibitory mediator, is a purely physical process controlled alone by the visco-elastic properties of the contractile elements

For a number of smooth muscles (intestine, 54, retractor penis, 243, nictitating membrane, 109, urinary bladder, 60), it has been demonstrated that the maximal tension increment produced by electrical stimulation or mediator action increases with the resting length of the muscle up to an optimal length, beyond which a decrease in tension increment occurs. This is exactly the same tension length relation as for skeletal muscle (183) As in the latter, at least for some smooth muscles, this optimal length is beyond the maximal physiological length The tension increase with length apparently does not apply for autogenous rhythmic contractions (urinary bladder, 161, 162, uterus, 211), probably due to a depressing effect of stretch upon the pacemaker Only one investigator has tried to determine the "absolute muscle force" (maximal tension increment per cm.$^2$ cross section at optimal muscle length) Ducret (73) gives a value of 800 gm./cm.$^2$ for the epinephrine contraction of mesenteric arteries Using tension and dimension values given by the authors or assuming the probable size of the muscle strips, the absolute muscle forces can be calculated as 650 gm./cm.$^2$ for the indirectly stimulated nictitating membrane (109), 630 gm./cm.$^2$ for the histamine

contraction of the cat ileum (54), 440 gm./cm.² for the electrically stimulated and 650 gm./cm.² for the epinephrine contracted retractor penis (243). These values are much lower than those for most striated muscles (89), but their conformity is astonishing. However, more data are needed to allow any general conclusion.

As in striated muscle, the tension increment of smooth muscle is affected not only by the muscle length but also by the experimental temperature (185, 244). Hydrostatic pressure affects the tension response of smooth muscles rather similarly to that of striated muscles, and very high pressure produces a compression contraction with a tension surpassing that for maximal electrical stimulation (74, 78).

"Tonus." The change in our general attitude towards the tonus problem as far as it concerns smooth vertebrate muscles is best illustrated by a comparison of two papers of Bozler, one published in 1928 (40), the other in 1936 (42). In the first paper he tries to demonstrate that also in vertebrate smooth muscles, the coarse and thin fibrils existing side by side in the same cell have different functions, tonic activity and quick contraction respectively. In the second and in later papers, he states emphatically that there does not exist any specific tonic mechanism and that tonus can be explained perfectly by the normally slow relaxation of smooth muscles, which can be speeded up at least for some smooth muscles by the action of an inhibitory mediator, a mechanism not existing in striated muscles. Bayliss (19) pointed out as early as in 1928 that, in consideration of the slow relaxation of smooth muscles, tonus can be maintained by continuous excitations with little expenditure of energy. The measurement of the heat production of vertebrate smooth muscles during so-called tonic contractions is a task which nobody familiar with the pitfalls of our present methods has yet felt inclined to attempt. For the relatively quick contractions of the cardiac sphincter of the turtle stomach following vagal stimulations, Snyder (218) found after applying various correction factors that the ratio of the heat to the product of tension, weight, and time is of the same order of magnitude as in striated muscles. Although there is no doubt that all our recent experimental results favor a unitary conception for the quick and the tonic contractions, the last word concerning the tonus problem of smooth vertebrate muscles has not yet been written.

Conclusions. Vertebrate smooth muscle organs show a large diversity of physiological characteristics. However, all phenomena closely associated with the contractile mechanism proper are common to all smooth muscles and are rather similar to those of striated muscle, the time factor alone is much shorter in the latter. While for striated muscles only one unitary mediator mechanism eliciting the contractile discharge exists, smooth muscles can be classified into two groups according to the excitatory mediator involved. Furthermore, for a number of smooth muscles there exists also an inhibitory mediator. For these muscles, the strength and the time curve of activity are determined by the spatial and temporal interaction of the two antagonistic mediators. For some smooth muscles, activity might be controlled by the combined action of more than two

physiological mediators Due to the smallness of a smooth muscle cell, the integration of the activity of the single cells into the function of the whole smooth muscle organ requires a more complicated mechanism than that for integration of single striated fibers into the activity of an anatomical muscle. Diffusion of the mediator is apparently only of minor importance under physiological conditions. In some smooth muscle organs there exists a mechanism for conduction Whether the latter occurs through protoplasmic bridges of the muscle substance proper or through the terminal nerve reticulum, cannot be decided yet, and difference in this respect might be present from one organ to the other There is no doubt that some smooth muscle cells are capable of autogenous impulse or pacemaker activity But this does not mean necessarily that all autonomic activity of smooth muscle organs is of myogenic origin The autonomic impulse mechanism of the cardiac ventricle comes into play, as a rule, only when the conduction system is interrupted Even striated muscle fibers show automatic impulse formation after denervation, but nobody would conclude that the impulses for normal skeletal muscle activity are myogenic. In the majority of the smooth muscle organs, activity is controlled and governed by extrinsic nerves and by the intrinsic ganglion plexi, thus probably suppressing any automatic impulse tendency of the muscle cells proper, if existent The correlation between autonomic nervous system, mediator action, and contractile mechanism is in some smooth muscle organs dependent on the balance between the levels of various hormones.

Since the contractile mechanism in smooth muscle is so similar to that of striated muscle, where it can be studied under much more favorable conditions, the main task for the future concerning the physiology of vertebrate smooth muscles will be the analysis of the mechanisms by which contraction of the single muscle cells are integrated into the specific functions of the various smooth muscle organs.

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# WATER EXCHANGE

JOHN P PETERS

*Department of Internal Medicine Yale University*

Some years ago the author (317) attempted a comprehensive treatment of the functions of water in biological reactions This article must lean heavily on that earlier volume and on previous reviews, confining itself largely to recent contributions

MEMBRANE EQUILIBRIA AND TRANSFERS OF WATER WITHIN THE BODY Despite the extreme differences in composition of the contents of its various compartments, a uniform osmotic pressure prevails throughout the fluids of the body It follows that the membranes between these compartments must universally permit the free passage of water and that exchanges of water among the compartments must be determined by the hydrostatic and osmotic forces within them

*Movements of water between the circulating blood and the extracellular fluid.* The demonstration, by direct analysis of edema fluid and other transudates and by inferential methods that the extracellular fluid has the composition of an ultrafiltrate of plasma, is more substantial proof of the validity of the Starling theory than are quantitative data which show that the colloid osmotic pressure of the plasma and the capillary blood pressure have the proper relative magnitudes (259, 382) The effective hydrostatic force tending to expel fluid from the capillaries is the capillary blood pressure minus the tissue pressure (i e., the elastic resistance of the extravascular spaces to distention), the effective osmotic force which tends to drive water back into the blood stream, the colloid pressure, is the osmotic pressure of the serum proteins minus the osmotic pressure of the proteins in the perivascular fluids

The colloid osmotic pressure of the proteins of normal plasma, including a contribution from the Gibbs Donnan effect, amounts to about 30 cm of water or 22 mm of mercury (436) The greater part of the colloid osmotic pressure derives from serum albumin, which has a smaller molecule than globulin The capillary blood pressure varies greatly according to its anatomical situation and local circulatory reactions In those parts of the general circulatory system that have been investigated, the pressure at the arterial end of the capillaries appears to be greater, that at the venous end less, than the colloid osmotic pressure (259)

The impermeability of the capillary walls to protein is not continuous, absolute or uniform McCarrell, Thayer and Drinker (297) have verified Starling's (382) observation that the concentration of protein in lymph from the liver is practically identical with that of plasma. If the interstitial fluid about the portal capillaries did not contain high concentrations of protein, exchanges of fluid in this region would be impossible because in the portal vein the blood pressure is lower than the colloid osmotic pressure of the plasma proteins Although the proteins in hepatic lymph may be partly formed *in situ*, the rapid

appearance in thoracic duct lymph of foreign protein (281) and of dyes that adhere to proteins (62, 127, 364), after intravenous injection of these materials, indicates that proteins must escape readily from the capillaries of some regions drained by the thoracic duct The low concentrations of protein in edema fluids and transudates, 0 06 to 0 5 per cent, (179, 259, 317, 347, 410) and the failure of dyes to enter these fluids in appreciable quantities (127, 160) denotes that the capillaries of peripheral tissues are far less permeable Foreign proteins and dyes also appear in smaller quantities in lymph draining the extremities than they do in thoracic duct lymph In the latter, for example, after injections of the blue dye, T-1824, Courtice (78) and Ferrebee et al (127) found dye concentrations as high as 30 to 40 per cent of those in plasma with a lymph flow of 100 to 200 cc per hour while in cervical lymph the concentrations were only 3 to 15 per cent of those in plasma with a lymph flow of only 10 to 20 cc per hour Anoxia (258, 291) and vasomotor disturbances induced by physical agents (253, 260) or drugs (39, 172, 384, 394) can increase capillary permeability so much that proteins escape freely from the circulation Fluid of urticarial wheals or angioneurotic edema contains high concentrations of protein (173, 347) Such lesions also take on an intense blue after injections of T-1824 (127)

Tissue pressure also varies with structure and location In the subcutaneous tissue of a normal subject in the recumbent position, at the level of the heart, it is usually less than 5 cm of water, occasionally as high as 7 to 8 cm (53, 293, 376, 431) It rises when venous congestion is induced, because of the expansion of the veins (431), and is also increased by accumulation of edema fluid (376) At rest in the recumbent position, Wells and Youmans (431) found intramuscular pressures from 2 to 11 cm of water, highest in muscles with tight fascial sheaths When venous pressure was raised to 100 cm the intramuscular pressure in the tibialis anticus rose as high as 50 cm of water The relatively small expansibility of muscles may explain the comparative immunity from edema that they seem to enjoy

*The volume of the circulating blood* The retention in the blood stream of dyes used to measure plasma volume depends not upon their molecular size or other inherent physical properties, but upon their propensity to combine with serum protein, especially the albumin fraction (331) Like the latter they are not entirely restrained by the capillary walls Large amounts of T-1824 or brilliant vital red appear in thoracic duct lymph shortly after either is injected into the blood stream (62, 127, 364) If they were steadily, gradually and permanently removed from the circulation by this route from the moment of their injection, the true volume of the plasma could be estimated by extrapolating to the moment of injection the curve describing the decrease of dye concentration in the blood plasma There is no certainty, however, that the dissemination of dye through the circulating blood and its escape into thoracic duct lymph are either initiated or reach steady states simultaneously The return of dye to the blood stream again via the thoracic duct defies correction The amount of dye returned may ordinarily be so small, as Courtice claims (78), that the error of including a shunt in the system is of no great significance Variations in the volume and flow of thoracic duct lymph, which may occur under the very conditions in which

knowledge of plasma volume changes is most desirable, must introduce incommensurable errors of no negligible magnitude if, as Ferrebee, Leigh and Berliner (127) have estimated, the thoracic duct lymph system may contain as much as 7.5 per cent of the dye injected As evidence that removal of dye from the blood stream is not a uniform process, Gilder, Müller and Philips (163) found that extrapolation of the disappearance curve to zero time shortly after mixing is complete and extrapolation at a later time yield different values for plasma volume

According to most observers the carbon monoxide method, which measures blood volume directly, yields smaller values than the dye method does (29, 365) In normal subjects Hopper (226) found that the two methods agreed on the average, but differed in one direction or the other in individual subjects It seems highly unlikely that some of the carbon monoxide is absorbed by myohemoglobin or that it penetrates to cells in repositories such as the spleen (226) It seems more likely that technical errors, all of which will tend to give excessive values, cannot be excluded and that, as Whipple and others (183, 365) have suggested, the red blood cells are not evenly distributed throughout the circulation Transfusions with red cells containing radioactive iron or phosphorus (62, 181, 182, 183) are not practical for most purposes

Both carbon monoxide and dyes appear to give too large values for blood volume in normal persons, which may be greatly and unpredictably magnified by physiological and pathological disorders. The carbon monoxide method, though technically more difficult, is probably less subject to gross errors because red blood cells are less prone than proteins and dyes to escape from the circulation (145, 226) Hopper (226) found that while the two usually agreed well in normal subjects and in many disordered states, the dye method occasionally indicated paradoxical expansions of the plasma when the carbon monoxide method and all other criteria indicated hemoconcentration

The most extensive series of measurements, by Gibson and Evans (161), by a refined technique with the blue dye T-1824, gave blood volumes for normal resting adults of 2990 to 6980 cc , averaging 4635 cc Related to weight the average was 72 4 cc per kgm with extremes of 46.3 to 97 7 cc Related to surface area, with which the correlation was somewhat closer, the average was 2750 cc per sq m There was a distinct sex difference, the average for males being 77 7 cc. per kgm (range 66.2 to 99 7), for females 66 1 cc per kgm (range 46.3 to 85 4) This difference is not as Chang and Harrop (61) earlier suggested, referable to differences in body build (161, 226) In the series of Gibson and Evans (161) the average for males was 2930, for females 2530 cc per sq m The greater volume of blood in males depends chiefly upon the larger proportion of cells In adults the ratio of blood volume to size varies little with age Immediately after birth infants may lose considerable fluid with concomitant hemoconcentration (19) which rapidly disappears when adequate fluids are taken In the first weeks of life the cell volume falls while the plasma volume remains constant (20) Both blood and cell volumes are greater in proportion to body weight, but smaller in proportion to surface area, in children than in adults (94)

*The lymphatic system* Comparisons of the compositions of the two media

can leave no doubt that lymph is an ultrafiltrate of blood plasma (13, 105, 210) The lymphatics appear to provide an alternative route by which fluids may be returned from the extracellular spaces to the blood stream They also seem to have the faculty, not possessed by the blood capillaries, of removing from the tissue spaces particulate matter and large molecules such as proteins and lipids Foreign proteins are not absorbed directly from the tissues into the blood stream, but find their way thither through the lymphatic channels (131, 268) unless the permeability of the blood capillaries has been impaired (132) Even larger particulate materials enter lymph channels from the interstitial spaces with relative ease, although they do not seem to be able to escape in the opposite direction (133, 227, 274) The fact that the concentration of protein is regularly higher in lymph than in the interstitial spaces is further evidence that colloids can enter but not leave the lymphatics The colloid osmotic pressure of the proteins that gain access to the lymph, aided by the tissue pressure, provides a force by which the lymphatics may be filled The flow of lymph from the extremities is accelerated by active contraction of the muscles (422, 435) which, aided by the valves in the lymphatic trunks, provides additional propulsive energy Lymph flow varies directly with the quantity of fluid in the extracellular spaces, presumably because this influences tissue tension and the quantity of protein in the interstitial fluids (209, 422) Filling of the lymphatic capillaries—and, to this extent, the flow in these vessels—is opposed by the force of gravity and by the colloid osmotic pressure in the interstitial fluid about these capillaries (96)

Concerning the absolute volume of the lymphatics there is no information Both the volume and flow of lymph in the thoracic duct increase when food and fluids are introduced into the gastrointestinal tract, when gastrointestinal secretions are stimulated and when fluids are injected intravenously (421)

*Interstitial fluid* From analyses of edema fluids (176, 177, 206, 273) and by direct sampling of extracellular fluid from muscles (290), it has been established that the interstitial fluids, like lymph, have the the characteristics of ultrafiltrates of plasma, differing from plasma only in containing smaller quantities of proteins and lipids The cations in such fluids consist chiefly of sodium, while the principal anion is chloride, distinguishing them from most intracellular fluids in which potassium and phosphate predominate The total quantities of sodium and chloride in the body could be accounted for if they were confined to a volume of plasma ultrafiltrate equal to 20 or 30 per cent of the weight of the body (199) After the injection of a number of substances, among them sulfocyanate, sulfate, sucrose (263), magnesium (374) and bromide (49,372,445), the concentrations of these substances in the plasma indicate that they are dispersed through a volume of fluid of the same order of magnitude The same is true of radioactive sodium (242, 281) and chloride (443) If solutions containing ordinary sodium or chloride are injected, the increments of these ions in the plasma indicate that the added ions are confined to a space of about the same size Analyses of muscles yield enough chloride and sodium to account for an extracellular volume of about 20 per cent (114, 115, 205, 290), which coincides

with the volume into which these ions will diffuse when muscle is suspended in salt solutions (112, 438)

There is, therefore, a differentiated body of fluid, amounting to from one-fifth to one-third of the body mass, which has the characteristics of an ultrafiltrate of blood plasma with which it is in diffusion equilibrium This body of fluid contains most of the sodium and chloride in the animal Individual tissues, on the other hand, contain quantities and proportions of sodium and chloride that cannot be attributed to such an extracellular ultrafiltrate (11, 126, 197, 199, 282, 283) The quantities of these constituents in cells vary from tissue to tissue, being relatively large, for example, in red blood cells, the cells of the gastric and intestinal mucosa and the kidneys (11, 283), almost negligible in muscle and liver cells (11, 126) These intracellular fractions of sodium and chloride do not appear to traverse cell membranes freely Radioactive sodium penetrates the extracellular fluid with great rapidity, but comes into equilibrium only slowly with the sodium in tissues that contain high concentrations of this element (242, 281) Amberson was able both *in vivo* (11) and *in vitro* (314) to wash chloride easily from muscle, liver and kidney cells, the concentration of chloride in these tissues when plotted against time, describing a curve that approached zero From other tissues with initially high chloride it could not be easily removed and the curves of concentration in these tissues approached not zero, but a positive value Part of the chloride in these tissues was restrained from escaping

Variation of the concentration of sodium in blood plasma, instead of changing the concentrations of sodium in the blood cells, causes water to move to and from the cells in such a manner that the osmotic pressures in the two media remain always identical (318) Similar reactions between tissue cells and the extra cellular fluid have been demonstrated by indirect methods (110, 112, 114, 115, 300, 458) From such experiments it has been estimated that all or almost all of the sodium in extracellular fluid must be osmotically active, that sodium salts are osmotically the most important components of this fluid, that they do not diffuse across cell membranes and therefore control the distribution between the intracellular and extracellular compartments of water, to which all cell membranes are permeable The impermeability of cells to sodium is not un conditioned sodium will enter even muscle cells when they become depleted of potassium (219, 220), but the transfer is probably not effected by diffusion alone

*The volume of the extracellular fluid* can be estimated only from measurements of the volumes of distribution of a number of substances that diffuse freely from the blood stream through the extracellular fluid, but are more or less completely excluded from cells At the end of an appropriate interval after administration of the test substance its concentration in the serum and the amount excreted are measured The volume of distribution, $E$, is then estimated by the formula $E = \frac{A - U}{S_w}$ in which $A$ = the amount of substance given, $U$ the amount excreted in the urine, and $S_w$ its concentration in the water of blood serum None of the test substances commonly used is completely excluded from cells The volume

of distribution of sodium is regularly larger than that of chloride (443) or bromide (424) The distribution of thiocyanate differs from all three It enters red blood cells, probably certain tissue cells, and fluids in the gastrointestinal tract (81, 263) Sucrose (and probably sulfate) are probably more nearly restricted to the extracellular fluid Neither enters blood cells nor appears in appreciable quantities in gastrointestinal fluids On the other hand, both are so rapidly excreted by the kidneys that it is hard to maintain adequate concentrations in the serum for a sufficiently long period, and correction for excretion is undesirably large if renal function is normal (263) In these respects thiocyanate, which is excreted slowly, has an advantage over other test substances which have been proposed The distribution of test substances is not the same in all species (242, 443)

Estimation of the volume of extracellular fluid from the increments or decrements of serum sodium or chloride, or both, produced by administration of a known amount of sodium chloride, requires the administration of such large quantities of salt in such high concentrations that the osmotic effects of the injected solutions themselves alter the distribution of water in the body Changes in the volume of the extracellular fluids can be estimated with reasonable accuracy from balances of these ions and their concentrations in serum by the equation, $\Delta E = \frac{B - E_1 (\Delta S)}{S_2}$, in which $\Delta E$ = the increase of volume, $E_1$ = the initial volume, $\Delta S$ the increase of concentration in the serum of test material, and $S_2$ its final concentration in water of serum This requires that some value for $E_1$ be assumed

In eight normal adults by radioactive sodium Kaltreider (242) found extracellular fluid volumes of 20 8 to 28 7 per cent of the body weight, averaging 24 8, while by thiocyanate with correction factors of Lavietes et al (263) they were 19 5 to 25 8 per cent, averaging 23 3, which agrees with values found by Lavietes (263) By sucrose volumes about 10 per cent lower were obtained by Lavietes 16 4 to 28 0 per cent, averaging 21 4 per cent of the body weight The volume in a given individual is far more constant than the volumes in a group of individuals and is characteristic of the individual (263) The ratio of extracellular fluid to body weight is smaller in obese than in thin subjects both in humans (242) and in dogs (199) This ratio also varies from species to species, being nearer 0 30 than 0 20 in dogs (199, 443) The proportion of total water as well as the ratio of extracellular to intracellular fluid diminishes with age from fetal to adult life (232, 457)

*Forces that vary the volume of extracellular fluid* Smith and Mendel (362) in 1920 showed that within a few minutes after intravenous injection of quantities of saline or glucose solutions equal to the total blood volume, the blood volume of rabbits had returned practically to its initial level and no gross accumulations of fluid could be found in the animals Similar observations have been made by numerous investigators (10, 340, 387) Ingested water is treated in much the same way (216, 328, 359, 360) The water is stored, chiefly in the interstitial spaces (205, 261, 387) Presumably the accession of fluid to the blood stream

increases the hydrostatic pressure and diminishes the colloid osmotic pressure enough to accelerate the current from capillaries to interstitial spaces Processes conducive to dehydration and hemoconcentration, conversely, detract from the volume of the interstitial fluid (115, 141, 294, 300, 310, 458) If the concentration of sodium salts in the body is increased the cells will yield water to the extracellular fluid because, since sodium does not penetrate cells, the effective or electrolyte osmotic pressure of the extracellular fluid rises (115, 318, 444) Vice versa, reduction of the concentration of sodium salts in the extracellular fluids causes the cells to expand at the expense of these fluids (110, 294, 299, 387, 458) There is every reason to believe that alteration of the osmotic pressure of cellular contents causes similar exchanges of water, but reactions of this type cannot be elicited by the mere administration of salts

Changes of the hydrogen ion concentration of the body fluids also cause shifts of water between cells and adjacent interstitial fluids, by reactions that have been most precisely defined by Van Slyke (411, 412) When acid is added to a biological medium containing protein the acid anion combines with base derived from the weakly dissociated protein to form a strongly dissociated salt This increases the number of osmotically active components in solution Acidification increases the osmotic pressure of cells more than it does that of extracellular fluid because cells contain more protein It therefore causes water to pass from extracellular fluid into the cells, while alkalinization moves it in the opposite direction Such exchanges of water, in response to changes of $CO_2$ tension, have been demonstrated in the blood both in the test tube (187, 218, 411, 412) and in the circulation (104, 218, 321), and in the muscles of living animals (114)

*Intracellular fluid and its control* Cellular membranes within the body appear to be generally permeable to water, hydrogen ions and probably ammonia, but to few other solutes In most animal forms there seem to be no barriers to the diffusion of urea (113), but in certain fishes uniformity of osmotic pressure is largely maintained by urea, which plays the rôle that in other animals is delegated to electrolytes (4, 366, 368) Glucose is distributed uniformly through the water of plasma and cells in the blood of man (250, 380) and certain other species, but is far less concentrated in the blood cells of rabbits and some other mammals (381) Direct proof that tissue cells admit glucose freely is hard to obtain because the sugar is so rapidly utilized by these cells (77) Solutes that can move unrestrainedly through cell membranes contribute to the total osmotic pressure of all the media to which they have access, but do not affect the distribution of water between media.

The formation of glycogen from glucose enables the cells to store large quantities of sugar in such large molecular form that the carbohydrate is effectually imprisoned in the cells and exerts a minimal osmotic pressure Lipids, because of their insolubility, must be osmotically almost inert. These intracellular colloids although they occupy considerable space, must exert a minor influence upon the distribution of water Protein would occupy a similar position were it not for its ability to combine with a great variety of other substances

Of chief concern from the standpoint of the internal water exchange is the

disparate distribution of electrolytes The almost complete exclusion of sodium from cells and the accumulation of potassium within them could not be achieved by processes of diffusion alone, it would require the expenditure of no mean amount of energy If the metabolic activities of blood cells are reduced to a minimum by chilling, no appreciable quantities of either sodium or potassium enter or leave the cells when the concentrations of these elements in serum are greatly increased or diminished (116, 318) Water passes in or out of the cells in response to such alterations of the concentrations of cations in plasma as if the sodium originally in the plasma and the added sodium or potassium were all osmotically active (116) When blood is placed in the incubator, so that the metabolism of the cells proceeds, potassium moves across the cell membrane, the direction of its motion depending not upon its concentration, but upon the nature of the metabolic activities of the cells (89) Inorganic phosphate manifests the same type of behavior (185) The energy required for these ionic transfers is presumably derived from the metabolic reactions with which their motions are associated The concentration of Na+K is greater in blood cells than in plasma Moreover, in the circulating blood the concentration of base in blood cells, unlike that in serum, can vary considerably without proportional transfers of water (116, 320) These and other observations (379) comprise a growing body of evidence that the inorganic components of cells are not all osmotically active as those of plasma appear to be

The general chemical patterns and behavior of tissue cells are similar to those of blood cells The cell membranes are not even freely permeable to the inorganic components that are particularly plentiful in cells Injected magnesium, for example, appears to be confined to the extracellular fluid (374) Inorganic phosphate penetrates muscle cells freely neither *in vitro* (109) nor *in vivo* (326) The volume of distribution of injected phosphate, though somewhat larger than the volume of the extracellular fluids, falls short of the total fluid of the body (312) The volume of distribution of potassium, immediately after its injection, is greater than that of the extracellular fluid, sometimes even exceeding the total fluid of the body Subsequently it is released from the cells in which it has accumulated, to be excreted by the kidneys (44, 437, 445) Although sodium is usually excluded from muscle cells, it will enter them in animals that have been depleted of potassium (219) Undoubtedly these inorganic materials contribute to the intracellular osmotic pressure, but not as inevitably as do the inorganic constituents of the extracellular fluids Elkinton, Winkler and Danowski (121) found that, although extracellular fluid changes could be estimated with accuracy from balances of sodium and its concentration in serum, total water changes could not be estimated with the same accuracy from the concentrations and balances of total base or of sodium + potassium It may be inferred that fractions of potassium, magnesium and phosphates in the cells are not osmotically active and consequently do not control water exchanges, presumably because they form undissociated or partly dissociated combinations with proteins This permits their concentrations to vary, within limits, in accordance with the activities of the cells, without obliging the cells to imbibe or eject proportional

quantities of water The passage of electrolytes and probably many organic compounds appears to be linked with metabolic reactions of these cells which provide the energy required to effect their transit

*The volume of the total and intracellular fluids* The volume of the intracellular fluids can be estimated only by subtracting the volume of the extracellular fluid from the total fluid By weighing animals before and after desiccation Darrow and his associates (199) have measured the total water in a number of species In rabbits this amounts to about 75 per cent of the body weight, in dogs to about 65 per cent, and in macacus monkeys to about 70 per cent The proportion of water is greatest in lean, least in adipose animals

As yet only one substance, heavy water, has been found which distributes itself rapidly and evenly throughout all the water of the body and which is not destroyed nor altered in the body By means of $D_2O$, Flexner, Gellhorn and Merrell (139) found 65 per cent of water in guinea pigs, Hevesy and Jacobsen (221) found similar amounts in rabbits In the guinea pig Flexner et al. (139) estimated that 73 per cent of the water of the blood was exchanged with the extravascular water in the course of a minute In the rabbit $D_2O$ was distributed throughout the extracellular fluid within 30 seconds and throughout all the water of the body within 30 minutes after its injection

By means of urea Painter (315) obtained reasonable values for the total water of dogs Small amounts of this compound may, however, escape recovery and large corrections must be made for its continual production in the body and its rapid excretion by the kidneys It has been shown that sulfanilamide, which Marshall (285) claimed had the desired properties, as well as other drugs of its group, form compounds with proteins and are most capriciously distributed through blood and tissues (118, 211) Thiourea has been tested by Purple and Lavietes and by Danowski (90) Although originally reported to be inert, universally diffusible and completely excreted (40), a small fraction is stored or destroyed in the body It appears to be more useful as a measure of changes in the volume than as a measure of the absolute volume of the total water (90)

The total water balance can be estimated with considerable accuracy by the metabolic method which is discussed below Estimations from balances of sodium and potassium and their concentrations in blood plasma have proved unreliable for reasons mentioned above (121)

Exchanges of water with the environment *Thirst.* The sensation of thirst does not seem to originate in dryness of the oral mucosa It is not affected by absence of salivary glands (385) or resection of the olfactory, gustatory or trigeminal nerves (32) Even in diabetes insipidus thirst can be slaked through a stomach tube (138), while a dog with an esophageal fistula will drink enough to meet its immediate needs (7) When their diets and water intakes are varied, dogs will drink in proportion to the water deficits they incur (341) Dill (100) has reported a burro that, after excessive sweating in tropical heat, drank without a pause 12 liters of water, replacing its fluid deficit with considerable accuracy Drinking is attuned not only to the volume of fluid in the body, but also to the concentration of solutes in this fluid Gilman (165) has suggested that the stim-

ulus to thirst is the water content of the cells After intravenous injection of hypertonic sodium chloride, dogs immediately consumed enough water to restore the osmotic pressure of the body fluids to its initial level, despite the fact that they already contained excessive amounts of water On the other hand, dogs depleted of salt by Darrow and Yannet (95) evinced no thirst despite hemo-concentration Dogs evinced less thirst after urea solutions than they did after salt solutions of equal osmotic strength (165) The influence of cellular hydration must be less than absolute, since animals or men who have been depleted of salt and water will drink enough water to reduce the concentration of salt in the body fluids below normal, and animals dehydrated by urea do manifest thirst

*Alimentary exchanges of water* In the middle of the last century Bidder and Schmidt (36) demonstrated that enormous quantities of water and salts are continually poured into the stomach and intestines, only to be again reabsorbed The similarity between these fluids and blood serum and the influence of this exchange upon the internal environment were vividly illustrated by Schmidt's (349) studies of epidemic cholera, dysentery and catharsis The full implications of these phenomena were recognized by Gamble (150, 152, 153), who showed that all the secretions of the alimentary canal and of the digestive glands, in spite of the diversity of their chemical patterns, have the same osmotic pressure as body fluids This has been verified not only by chemical analysis, but also by measurements of vapor pressure (167) The osmotic pressure of the gastric (167) and pancreatic (21) secretions varies with that of the serum

Except in the acid secretions of the stomach, where it is largely replaced by the hydrogen ion, sodium is the predominant cation of these fluids, while the anions consist almost entirely of chloride and bicarbonate (31), unmistakable evidence that the secretions are derived from extracellular fluids

Solutions introduced into the alimentary canal become isotonic with blood serum during the process of absorption This adjustment is accomplished by excretion of water and salts and the simultaneous absorption of solutes, if the latter can be absorbed A hypertonic solution of glucose increases in volume by the addition of salt and water until it reaches the osmotic pressure of serum A hypotonic solution loses volume, although it gains salt, until it reaches the same pressure But even while these solutions are gaining or losing fluid, glucose is being withdrawn from them into the blood (146, 275, 330) The actual quantity of fluid excreted in response to the ingestion of food or fluid is determined by the osmotic pressure of the ingesta The amount of water ultimately left in the normal gut depends upon the quantity of solutes which is not or cannot be absorbed Water or solutions containing completely absorbable solutes may be entirely absorbed, but before this process is terminated the solution is replaced by a salt solution having a composition characteristic of the secretions of the gut (55) If an ion, like sulfate, which cannot be easily absorbed, is introduced, little salt enters the gut and what does enter is eventually almost completely reabsorbed, leaving an isotonic solution of sulfate salt (97, 228, 416) In the stomach secretion follows the same principles as it does in the intestine, but absorption (75, 117), even of water (74), is almost negligible In

the colon absorption predominates, this is the site of the final concentrating process that yields the semisolid feces (12, 171)

The alimentary canal affords little protection to the internal environment Absorption of water and salt does not cease when there is a plethora of these materials in the body Secretory activity continues when the salt concentration and volume of the body fluids is depleted (248, 269, 378) Since these secretions are reabsorbed this has no vicious effect so long as the alimentary canal, kidneys and other organs are functioning efficiently If, however, the gastrointestinal contents are removed by vomiting, diarrhea, by a tube or through a fistula, the uncontrolled secretory activity may lead to disastrous dehydration and salt depletion

The mass movements of fluid involved in the processes of secretion and reabsorption require some other force than osmotic pressure Wells (427, 428, 429) believes these movements are controlled by a balance between the colloid osmotic pressure of the proteins in the tissue fluids of the intestinal villi which tends to withdraw fluid from the intestine, and the hydrostatic pressure on the two sides of the intestinal mucosa This equilibrium is defined by the equation

$\frac{dw}{dt} = K(TP - OP_t - IP)$, in which $\frac{dw}{dt}$ = the rate of passage of fluid into the intestine, $TP$ = the hydrostatic pressure in the tissue spaces of the villi, $OP_t$ = the colloid osmotic pressure of the fluid in these spaces, and $IP$ = the intraintestinal pressure. $TP$ is a product of the state of congestion of the blood vessels in the intestinal wall and the distention of the lacteals Wells found that the introduction into the intestines of food and fluid regularly evoked circulatory congestion, which disappeared when absorption began (430) He has estimated that the magnitude of the various forces involved meets the requirements of the equation and has shown by a large series of experiments that the direction and rate of flow of fluid across the intestinal wall vary with $TP$ and $IP$ as theory demands (429)

*Loses of water through skin and respiratory passages* The insensible perspiration, water constantly lost by evaporation from skin and respiratory passages, must depend upon the differences in temperature and water vapor pressure between the surface from which evaporation takes place and the environmental atmosphere During normal respiration, with the atmospheric temperature between 23° and 25°C the aqueous tension of alveolar air is about 2 mm Hg lower than it would be if the air were completely saturated at rectal temperature As the environmental temperature rises to 37 5° to 41° the vapor tension of the alveolar air also rises Saturation is more nearly attained during a period of apnea, alveolar vapor pressure falls during hyperventilation (68) The insensible water from the skin seems not to emerge through the skin to evaporate on the surface, but to escape as water vapor directly from within the skin, which is permeable to gases, but not to water (289) So long as the sweat glands are inactive the insensible perspiration is almost, if not altogether, devoid of solutes (33, 147 188, 413, 415)

*Metabolic measurement of water balances* Vaporization from skin and respiratory passages is one of the chief processes by which heat is eliminated from the body When heat production is moderate and when environmental temperature and humidity are equable, approximately 25 per cent of the heat produced by a normal individual is lost by evaporation Newburgh (234, 311) proposed that this relation be used to estimate water balances in the following manner The water balance, $WB$, may be defined by the equation, $WB = (W_2 - W_1) + (S_e - S_i) + (C + 0.54P + F)$, in which $W_2$ and $W_1$ are the final and initial body weights, $S_e$ and $S_i$ are the solids of excreta and ingesta, while $C$, $P$ and $F$ are the weights of carbohydrate, protein and fat burned by the subject The first two terms, the weight gained and the solids lost, may be measured directly with considerable accuracy The last term, the quantity of food burned, can be estimated only by indirect methods that involve a series of assumptions that $C$ is equal to the carbohydrate eaten, that $P$ can be calculated from the nitrogen excreted in the urine, and that the insensible loss bears a fixed relation to the heat production (for details of methods of calculation see (262, 324))

With atmospheric conditions accurately controlled in normal subjects under basal conditions, insensible loss is a reasonably accurate measure of heat production Any change of body temperature, since it signifies that heat loss and heat production are not equal, introduces an error Exercise—even such slight exercise as shivering—may disturb the normal relations between evaporation and heat production At temperate or high temperatures the burden of heat dissipation imposed by exercise falls entirely upon evaporation (106, 107, 192) Sensible perspiration invalidates the procedure because, when sweating begins, the proportion of heat lost by evaporation mounts rapidly (106, 450) Moreover, water lost from the skin as sweat may escape evaporation Nevertheless, in normal subjects, if environmental temperature and humidity and physical activities are kept within such limits that sweating and shivering are eliminated, the relation of vaporization to heat production is remarkably constant (234, 265, 311) In a series of studies by Newburgh et al (311) heat production, estimated from insensible loss, the carbohydrate of the diet and urinary nitrogen, differed by less than 5 per cent from indirect calorimetric measurements when averages of several 24-hour periods were taken Greater discrepancies were encountered in shorter periods of observation (234, 311)

In febrile diseases the method cannot be used In certain other diseases and disorders the proportion of heat lost by vaporization may be abnormal (86, 164, 237, 262, 377) In hyperthyroidism vaporization appears to be relatively increased, probably because sweating is seldom completely abolished (86, 237) In myxedema an unusually small proportion of heat is lost by evaporation (164) A number of observers have detected no changes of insensible perspiration after oral or parenteral administration of moderate (69, 239, 266, 267) or even considerable (184) amounts of water or isotonic salt solution Patients with established edema appear to have normal insensible losses (238, 249, 377) On the other hand, the simultaneous administration of excessive amounts of water and pituitrin, which greatly increases the load of water in the body, seems

to exaggerate vaporization (184, 280, 339), while severe dehydration diminishes it (5, 184, 280, 444) Injection of hypertonic salt solution also decreases it (170) Gilman and Barbour (166) reported that insensible losses vary with the osmotic pressure of the body fluids Although osmotic pressure must influence vaporization the large variations of insensible loss noted by Gilman and Barbour cannot have been referable directly to changes of osmotic pressure, since the difference between the vapor pressures of blood and distilled water amounts to only about 0.3 per cent of the total vapor pressure of such solutions at body temperature.

*Sensible perspiration (sweating)* When the environmental temperature, humidity or heat production rises so high that the necessary amount of heat cannot be eliminated by the usual processes, the sweat glands are brought into play In the nude resting subject at moderate humidity sweating becomes perceptible at about 31°C, but clothing or even slight muscular activity will bring it on at 25° to 28° (106, 191, 194, 450) When the temperature exceeds about 35° the heat lost by evaporation exceeds the heat produced, because radiation no longer eliminates heat from, but contributes it to, the body (106, 449) Subjects studied by Adolph and Dill (6, 8) at Boulder City, at a mean shade temperature of 35.3°C drank an average of 4000 cc of water daily, although they drank only 1400 cc in Boston during the winter Voluntary intakes as high as 13,600 cc of water per day have been recorded During exercise one subject eliminated 1700 cc of water in an hour Respiratory losses of water increase only slightly with temperature in man, since humans do not augment the respiratory minute volume appreciably in behalf of temperature regulation (6) The dog, lacking sweat glands, resorts to panting to eliminate excessive quantities of heat Since this mechanism is less efficient than sweating, the dog adapts less effectively than man to extremely hot atmospheric conditions (101)

Women have the ability, not shared by men, to reduce heat production at moderately high temperatures, thereby delaying the onset of sweating (193) Persons who are partially or totally deficient of sweat glands are unable to prevent the body temperature from rising in hot climates, especially when they exercise (140, 338), their insensible loss is, however, quite normal (338) During the first few days of sojourn in a hot climate the quantity of sweat produced in response to a given stimulus increases, while the stimulus required to initiate sweating and the interval between stimulus and response diminish (448, 449) This is part of the process of acclimatization to the tropics

Both insensible perspiration and the secretion of sweat appear to be regulated by the amount of blood circulating in the skin (194, 449) The controlling factor cannot be the temperature of the skin itself, because this may diminish as a result of evaporation if sweating is profuse Winslow and Gagge (448) have suggested that sweating may be controlled by the internal (rectal) temperature, which increases slightly under conditions that promote sweating even when the skin temperature falls A rise of internal temperature even without change of environmental temperature or humidity, will induce sweating

Sensible perspiration differs from insensible in carrying with it solutes, among them electrolytes, chiefly sodium and chloride (102, 103, 136, 188, 225, 294, 295, 306) This marks its origin from extracellular fluids As the rate of sweating increases the concentrations of sodium and chloride rise (102, 188) The ratio of chloride to sodium in sweat is not, as it is in extracellular fluids, about 1 4 1 0, but approximately 1 1 (102, 295) Sweat is distinctly hypotonic, the average concentrations of Na and Cl in the sweat of males being only about 30 mM per liter, varying from 10 to 70 mM (102, 295) Some persons habitually lose more sodium and chloride per unit of water than others (102, 295) without relation to the concentrations of these ions in their sera (102) Women regularly lose less than men do (295)

As sojourn in a hot climate is prolonged the salt content of sweat diminishes (73, 102, 295) The capacity to adapt in this manner to prolonged or frequent exposure to heat varies from individual to individual (102, 295) It is relatively easy to deplete some persons of salt by repeated sweating, others, especially women, suffer little depletion despite strenuous measures (295) There is a suggestion in McCance's (294) experiments that the reduction of salt in sweat during adaptation to heat may depend upon the development of a certain degree of persistent salt depletion, since it could be prevented by administration of abundant quantities of salt Forbes, Dill and Hall (141) noted that the average volume of interstitial fluid of men fell somewhat when they moved to a hot climate, an indication that their bodies contained less sodium and chloride Sweating *per se* tends to withdraw more water than salt from the body, but it does, in the unacclimatized subject, cause salt depletion If only water is taken to replace the lost fluid the concentrations of sodium and chloride in extracellular fluids fall (225, 306)

Perspiration, insensible and sensible, is attuned to the regulation of temperature rather than body water Insensible loss does diminish slightly in severe dehydration and when the concentrations of sodium and chloride in extracellular fluid rise inordinately Men exposed to high temperatures sweat less when deprived of water than when they are allowed to drink freely (178) Their temperatures consequently rise There is no evidence that sensible perspiration is promoted by drinking unnecessarily large amounts of water (8)

*Measurement of water balances in sweating subjects* offers peculiar difficulties since water of vaporization no longer bears its usual relation to heat production In a large proportion of conditions, especially when sweating is profuse, an error in the evaluation of fat burned, which is the factor derived from insensible loss, is of relatively little significance in the calculation of the water balance Errors that may arise from changes of body temperature are also comparatively small Estimation of water balances from balances and concentrations in serum of sodium and chloride are totally invalidated by sweating

Regulation of body fluids by the kidneys Chief responsibility for maintenance of the volume and composition of the body fluids devolves upon the kidneys The elimination of abnormal substances and superfluous amounts of normal constituents is no more important than the conservation in proper

proportions of essential components, including water There can no longer be reasonable doubt that these objects are achieved by a combination of filtration, reabsorption and secretion Ingenious methods for the measurement of these three processes in the intact animal, all depending upon the clearance principle, have been devised, chiefly, by Smith (367) and his associates

The term *clearance*, originally introduced by Van Slyke (17), represents the virtual volume of plasma cleared of a given solute in a standard time Clearance $= \frac{UV}{P}$, in which $V$ is the rate of urine flow, while $U$ and $P$ represent the concentration of the test solute in urine and plasma respectively The clearance of any substance that is completely extracted from the blood plasma in its passage through the kidney measures the flow of plasma through the kidney A substance that has the properties required for measurement of renal plasma flow has been found in diodrast (370) The clearance of any substance that passes the glomerular filter freely, but is neither reabsorbed nor secreted by the tubules, will equal the rate of glomerular filtration. In man and most animals inulin (334, 367), a number of hexitols such as mannitol, sorbitol and dulcitol (375), and sucrose (368) have the properties required for measurement of filtration In the dog creatinine, first proposed for man by Rehberg (332), may also be used The difference between the filtration rate and the rate of excretion of the fully elaborated urine gives the rate of reabsorption of water The rate of secretion or reabsorption of any solute may also be measured by comparing its clearance with that of one of the purely filtrable solutes

By these methods Smith (368) has estimated that the average renal blood flow of an adult male amounts to about 1200 cc per minute, plasma flow about 700 cc. per minute. The average rate of glomerular filtration is about 130 cc per minute, the blood plasma losing more than 15 per cent of its water in its passage through the glomerular capillaries Since the normal rate of urine flow is from 0 7 to 1.5 cc per minute, 99 per cent or more of the water filtered through the glomeruli is ordinarily reabsorbed in the renal tubules

In amphibia and other lower forms of life the osmotic pressure of the urine never exceeds that of the blood The tubules of the mammalian kidney, by acquisition of the loop of Henle and the terminal segments of the renal tubules (383), can reabsorb water against the force of osmotic pressure, to produce a urine with an osmotic pressure far above that of plasma It has been shown by direct analysis that in amphibia (419) and in mammals (418), in its passage through the proximal tubules glomerular fluid loses all its reducing substances and a large proportion of its water and salt, leaving a solution with an osmotic pressure equal to that of the blood, but with a greatly altered pattern. Shannon (354) has suggested that in the proximal tubules glucose and other constituents with 80 per cent of the filtered water are withdrawn by a process of obligatory reabsorption, in the loop of Henle and the distal convoluted tubules electrolytes and water are further reabsorbed to establish the ultimate concentration and reaction of the urine by a facultative process

In the mammal the concentrating power of the kidney—i.e., the power to

reabsorb water against osmotic pressure—does not seem to be a single function limited specifically by osmotic pressure It has been shown by several observers (3, 154, 155, 169) that the limiting osmolar concentration for urea is greater than the limiting concentration for sodium chloride and that the addition of either one does not limit the concentration of the other Urea and sodium chloride do not compete with one another for water On the other hand, the concentrations of chloride, bicarbonate, sodium, potassium, inorganic phosphate, glucose and creatinine seem to be limited by the aggregate osmolar concentrations of these constituents, which do, therefore, compete with one another for water (154, 155) The excretion of water, then, depends not only upon the quantity, but also upon the nature of the solutes requiring elimination

Urea is always more concentrated in urine than in plasma, its clearance at mean rates of urine flow being about one-half as great as that of inulin In profuse diuresis the clearance rises slightly, in extreme oliguria it falls, the concentration ratio $\frac{U}{P}$ approaching an asymptote Since the capacity of the kidney to reabsorb urea is limited, the quantity of water preempted by urea for the urine is determined by the quantity of urea filtered which, in turn, must depend upon the rate of protein catabolism The diuretic effect of urea does not begin only after maximal concentrations have been reached, water and urea are proportioned to one another at all rates of excretion of urea (154, 155) In studies of water deprivation by Winkler, Danowski, Elkinton and Peters (442), the excretion of water by dogs and men varied directly with protein catabolism, although urinary concentrations of nitrogen never reached maximal values Nevertheless, as water becomes more precious less and less is yielded for each increment of urea up to the limiting concentration Urea clearances fall, accounting for the nonprotein nitrogen retention of dehydration As the quantities of urea requiring excretion diminish the volume of urine does not fall proportionally because the influence of other solutes persists For this reason it is impossible to obtain urine of maximal concentration from subjects with low protein catabolism by restriction of water (304)

Always more water than urea is absorbed, but sodium, chloride and water can be reabsorbed in the most variable proportions in a manner that seems, on the whole, contrived to preserve the volume and electrolyte patterns of the body fluids From experiments of Walker, Bott et al (418) 80 per cent of the water and about 60 per cent of the chloride of glomerular filtrate appear to be reabsorbed in the proximal tubules, in the loop of Henle further salt is withdrawn, the final abstraction of water is accomplished in the distal convoluted tubules Shannon (355) has suggested that the dilute urine of diabetes insipidus may be regarded as filtrate which has been subjected to all these processes except this final abstraction of water If a large amount of salt remains unabsorbed the power to reabsorb water is limited accordingly, but water may be excreted with little or no salt if the terminal concentrating process is inhibited

If large amounts of water are given without salt the resulting aqueous diuresis may carry with it from the body a certain amount of sodium and chloride (87,

388), but chloride excretion may even diminish slightly after moderate amounts of water (111) Since ingestion of water tends to lower the concentration of salt in serum and in glomerular filtrate salt should be more completely reabsorbed unless sufficient water were given to increase glomerular filtration (353)

If salt is eliminated from the diet or if the salt content of the extracellular fluids is depleted by sweating or vomiting, urinary chloride excretion promptly diminishes to the vanishing point A given quantity of water is excreted more slowly by an animal or man who has been depleted of salt than by a normal animal or man (296, 439) This delay in excretion may arise from several factors 1, circulatory depression that regularly attends salt depletion (310), 2, diminished glomerular filtration that may depend upon the circulatory depression (296, 439), 3, relative or absolute reduction of the volume of the extra cellular fluids The last results from the general tendency not to retain water without a proportional amount of salt and the transfer of water from the extra cellular fluids to the cells because of the reduced osmotic pressure of the former

Excessive amounts of salt or hypertonic salt solution provoke diuresis that may lead to dehydration (240, 444) As the concentrations of sodium and chloride in the serum rise, not only does the excretion of salt increase, but also its reabsorption, albeit to a lesser degree The concentrations of sodium and chloride in the serum consequently remain elevated or even increase (120, 190, 444, 456) This retention of salt has a double advantage. Displacement of salt from the urine permits other substances that compete with salt to be excreted with greater economy of water At the same time the accumulation of sodium and chloride in the body, by raising the osmotic pressure of the extracellular fluids, withdraws water from the cells, which thereby share in the dehydration which would other wise be borne entirely by the extracellular fluids

This reaction is observed not only after excessive doses of sodium chloride, but in most states of dehydration that do not originate from primary salt depletion When either dogs (119) or men (442) are deprived of water, as dehydration becomes advanced, sodium and chloride in the urine fall to a minimum, while their concentrations in the serum rise If urea is given with large quantities of water enough sodium chloride may be washed out in the diuresis to lower the concen tration of serum chloride (276) If dehydration is induced by giving urea with inadequate amounts of water, the concentrations of sodium and chloride in the serum rise (120) When large quantities of sodium sulfate are injected into dogs chloride mounts rapidly (351) Addition of sodium chloride to the sodium sul fate does not increase chloride excretion, but only accelerates the elimination of the sulfate, raising the concentrations of sodium and chloride in the serum still further Inorganic phosphate tends in the same manner to displace chloride from the urine (304, 446)

Potassium is eliminated at a somewhat faster rate than sodium and, instead of suppressing the excretion of the latter, causes a transitory sodium diuresis (351, 440) The reason for this may be that a large proportion of any surplus potassium is immediately taken up by cells, leaving its associated ion to combine with sodium in the extracellular fluids

Glucose which escapes reabsorption in the tubules requires for its excretion the same amount of water required by an osmotically equivalent quantity of salt (154, 155) It does not, however, tend to displace sodium and chloride from the urine On the contrary it accelerates their excretion to such an extent that it may deplete the body fluids of salt (15, 196, 247) Glycosuria is, therefore, peculiarly dehydrating

The final concentration of the urine depends upon the quantities in the glomerular filtrate of two groups of components which can be concentrated independently Maximal economy of water can be attained only if the two are excreted in optimal proportions One of these, of which urea is representative, can be reabsorbed to a limited extent, but is always more concentrated in urine than in blood plasma The quantity of water which it withdraws is, therefore, determined almost entirely by its concentration in the body fluids and the rate of glomerular filtration, it can be little influenced by tubular activity The other group is composed of a large number of solutes which resist the reabsorption of water in proportion to their aggregate osmolar concentration The only representatives that usually appear in large enough quantities to have an important effect upon the reabsorption of water are the inorganic ions of which sodium and chloride form the bulk The effect of these two on the volume and concentration of urine is not obligatory like that of urea, because they can be reabsorbed almost completely even when their concentrations in blood plasma are abnormally high They yield their places in the urine when there is need for conservation of water, either because there is a direct deficiency of this commodity or because it is needed for the elimination of other substances This permits these other substances to be excreted more rapidly and with greater immediate economy of water It also provides extra water for their excretion by extracting it from the cells Concentration, therefore, is not equally affected by all solutes, nor can it be regarded entirely from the standpoint of the ultimate composition of the urine, it must be viewed also in relation to the internal environment

Species differ in their ability to reabsorb water from the renal tubules against the osmotic pressure of solutes In this respect the kidney of the dog is far more efficient than that of man (208)

Nervous control of urine formation Marshall and Kolls (286, 287) demonstrated that injection of adrenalin and stimulation of the splanchnic nerves caused oliguria, while section of the splanchnic nerves and denervation of the kidneys induced diuresis The effects of splanchnotomy were neutralized by partial occlusion of the renal artery (288) When Richards and Plant (335, 336) maintained a constant flow of blood through the kidney, adrenalin and splanchnic stimulation had a diuretic effect The nerves of the kidney, therefore, appear to exert their principal action upon the renal circulation The negative results of Rhoads, Van Slyke et al (333) must be discounted because the animals used had been subjected to unilateral nephrectomy From investigations of the action of adrenalin, theophylline, artificial fever and spinal anesthesia on renal function of normal men (63, 369, 370, 371) by clearance techniques, Smith (369) concluded that filtration is regulated almost entirely by reactions

of the efferent arterioles By this arrangement filtration could be kept relatively constant in the face of changes of blood flow and blood pressure, reduction of blood flow by constriction of the efferent artery would be compensated automatically by an increase of filtration pressure From a re-evaluation of Smith's data and calculations, Lamport (256, 257) has concluded that glomerular filtration is controlled by activity of both afferent and efferent arteries, which seems more in keeping with other observations in the literature.

Adrenalin and splanchnic stimulation do not affect the excretion of all solutes equally and therefore presumably affect reabsorption as well as filtration (286, 287) The completely isolated kidney excretes a urine in which all the components are extremely diluted (27, 28, 383) Its loss of concentrating power is chiefly referable, not to lack of nervous control, but to absence of the posterior pituitary hormone (27, 383, 414) The renal nerves may, however, be implicated in the action of this hormone Bayliss and Fee (27) found that the polyuria characteristic of the isolated heart-lung kidney preparation did not develop, if the kidney was perfused *in situ* with a heart lung preparation from another animal, until the nerves to the kidney had been cut Posterior pituitary preparations do not check, but may even provoke, diuresis in animals anesthetized by ether, urethane and a number of other drugs (9, 52, 80, 305) Bayliss and Brown (26) found that ether anesthesia did not affect an explanted kidney, though it inhibited in a normal kidney polyuria induced by decerebration or hypophysectomy Anesthetics, therefore, appear to prevent variations in the concentration of the urine by acting through the nerves of the kidney (214)

The oliguria induced by emotional disturbances must probably be ascribed to posterior pituitary, not nervous, activity since it cannot be abolished by denervation of the kidneys and elimination of adrenal medullary activity (345)

THE ACTION OF ENDOCRINE GLANDS *The hypophysis* It was long recognized that diabetes insipidus could be produced less consistently by removal of the hypophysis (243) than by injury to adjacent portions of the brain (18, 45, 84, 243) Nevertheless, whatever its origin, the polyuria could be abolished by injection of extracts of the posterior lobe of the pituitary gland (60, 67, 307, 339, 425, 426) This paradox has been explained by two discoveries first, that the diuretic effect of removal of the posterior lobe of the hypophysis depends upon the preservation of some anterior lobe substance (392), secondly, that the secretory activity of the posterior lobe is controlled by the supraoptic nuclei in the hypothalamus (137, 230, 231, 278) Destruction of these nuclei or the tracts that connect them with the posterior lobe of the pituitary causes diabetes insipidus by inhibiting the secretory activity of this lobe

The antidiuretic activity of the posterior lobe appears to reside in or with the pressor principle, pitressin (224, 339) According to Fraser (144) the oxytocic principle has a diuretic action in the rat. Heller (214) has succeeded in freeing the antidiuretic factor of pressor activity Because diabetes insipidus develops only if there is some active anterior lobe tissue, it has been suggested that this lobe produces a diuretic hormone (22, 35, 433) The posterior pituitary cannot be merely an inhibitor of the anterior lobe since it acts upon the isolated kidney

Biasotti (35) believes the diuretic principle of the anterior lobe is identical with the thyrotropic principle. Schweizer, Gaunt et al (352) attribute the absence of polyuria after hypophysectomy to the failure of pituitaryless animals to eat. Winter, Sattler and Ingram (455) have shown that experimental diabetes insipidus of animals is minimized or abolished by starvation. Schweizer and her associates found that at the cessation of the initial self-terminative polyuria that followed hypophysectomy the experimental animals ate almost no food and lost weight. When they were given anterior lobe extracts appetite and polyuria returned simultaneously.

Although the posterior pituitaries of all vertebrates, including birds, amphibians, and teleost and elasmobranch fishes, possess antidiuretic principles (214), only the mammalian kidney reacts to these (54, 214). For this reason it has been inferred that it promotes reabsorption of water in the loop of Henle and the terminal segments of the renal tubules, which first appear in mammals. In frogs posterior pituitary extracts have effects of a different nature (46, 47, 214). The antidiuretic principle does not interfere with absorption of fluid from the gut nor its distribution in the body (216, 360). Insensible loss increases when pituitrin is given together with large amounts of fluid, but this probably derives from the overhydration rather than the effects of pituitrin *per se* (216, 280).

The primary defect in diabetes insipidus appears to be polyuria, not polydipsia, since in this condition thirst is abolished by nephrectomy (389, 390) and since water deprivation causes dehydration (138, 355). In hypophysectomized dogs (434) and patients with untreated diabetes insipidus (441) greatly reduced diodrast clearances have been reported. These Winer (441) attributes to disturbance of the secretory activity of the tubular cells rather than diminution of renal blood flow. Glomerular filtration is not altered in diabetes insipidus (59, 327, 355, 434). The transitory reductions of renal blood flow (189, 434) and glomerular filtration (434) immediately after injections of pituitrin are probably referable to vasopressor action of this extract. Its antidiuretic action persists after blood flow and filtration have again become normal (26, 356, 434). The most characteristic disorder of diabetes insipidus, experimental or spontaneous, is a defect in the tubular reabsorption of water that leads to the passage of large amounts of extremely dilute urine (54, 189, 325, 358). The dehydration is constantly combatted by ingestion of large quantities of water. Since both drinking and urination are intermittent functions the concentrations of solutes in the blood plasma are unusually variable. After administration of water the conductivity of the serum falls as it does in normals (135). After complete withdrawal of fluid (355) or administration of salt (70, 196, 355) serum sodium and chloride rise more rapidly than usual. This has given rise to the opinion that failure to reabsorb water is attended by excessive reabsorption of salt. The responses of the animal with diabetes insipidus to administration or deprivation of water are qualitatively similar to those of a normal animal, but are exaggerated by the extreme compulsion. The animal is in a continuous state of mild water depletion because drinking tends to fall somewhat behind urination. At the onset of experimental diabetes insipidus considerable sodium and chloride are

excreted before equilibrium is established (454) Although the concentrations of sodium and chloride in the serum rise quite early if water is completely withdrawn, some salt is lost in the urine by the continuing diuresis (355) Urea clearances are high in proportion to creatinine clearances in diabetes insipidus just as they are when the rate of urine formation is greatly accelerated by the administration of water (59, 233, 327) When the water intake is limited the volume of urine does not fall with the usual rapidity nor does its concentration rise to the usual extent (355) Dehydration acquires a greater intensity earlier After only 24 hours of water deprivation a dog with diabetes insipidus, studied by Shannon (355), had lost about 40 per cent of its body water and its serum sodium had risen from 145 3 to 175 5 mM per liter, a degree of dehydration that it would have required several days to produce in a normal dog The polyuria of diabetes insipidus is exaggerated by sodium chloride, but the concentrations of sodium and chloride in the urine do not rise so high, while those in serum rise higher than usual (70, 264, 355) Chu, Lin and Yu (70) found that the administration of salt increased the excretion of potassium and nitrogen, a response noted by Winkler, Elkinton et al (444) when the concentrations of sodium and chloride in the extracellular fluids rose excessively Hare, Hare and Phillips (196) have shown that the ratio, concentration of chloride in reabsorbed fluid concentration of chloride in serum, does not fall after administration of hypertonic salt solution in diabetes insipidus as it normally does This does not mean that sodium and chloride are more completely reabsorbed, but that the salt that is not reabsorbed demands more water for its excretion

The degree of polyuria also varies directly as the nitrogen excretion (453), the kidneys in diabetes insipidus can concentrate urea no better than they can salt When the polyuria is checked by starvation the specific gravity of the urine remains low The relative oliguria can be attributed to reduction of the solutes requiring excretion The concentration of urine will rise but not to the normal degree under the influence of extreme dehydration (355)

When active posterior pituitary extracts are given to animals or persons with diabetes insipidus the flow of urine falls sharply, while its concentration rises, the excretion of sodium and chloride diminishes (264 303, 426, 454) and the urea clearance falls (59, 233, 327, 355) Equilibrium is, however, established after a short interval (454) When pituitrin is given to a normally hydrated or a dehydrated animal the rate of urine formation is not appreciably altered (9, 52, 88, 307, 325, 339) When it is given with a dose of water the diuresis which would ordinarily result is abated or suppressed The effect of a given dose of pituitrin is inversely proportional to the amount of water that is given (325) It is doubtful whether the antidiuretic hormone actually accelerates the excretion of salt as some claim (358, 363, 408, 454), it does seem to check the excretion of water without altering the rate of elimination of sodium and chloride (88, 339) It therefore exaggerates the hemodilution that normally follows the ingestion of water (9, 52, 339, 360) Its antidiuretic action varies directly as the amount of water and inversely as the amount of salt given (9, 52, 80 298, 307) Melville (301) claims that posterior pituitary preparations have a positive diuretic effect

on animals which have been loaded with salt When, however, Adolph and Ericson (9) gave pituitrin with varying amounts of water, but a constant dose of salt, the volume of urine excreted was always the same Enough water was eliminated in every instance to permit excretion of the salt in less than maximal concentration, the remainder was reabsorbed under the influence of the pituitrin

Gilman and Goodman (168) demonstrated in the urine of dehydrated rats material resembling the antidiuretic principle of the posterior lobe They suggested that secretory activity of the posterior pituitary is automatically regulated in conformity with the water content of the animal Although this observation was abundantly confirmed (186, 213, 231, 417), antidiuretic material was not found in the urine of other mammals and the identity of the material in rat urine with posterior pituitary hormone was challenged (186, 417) The demonstration of antidiuretic activity in the urine of dehydrated dogs (195) and cats (231), its absence from the urine of dogs (195) and cats (231) with diabetes insipidus, and its presence in the urine of animals that have received injections of posterior pituitary extracts (215, 231, 236) have vindicated Gilman and Goodman's theory

*The suprarenal glands* Adrenalin, according to Kaltreider et al (241) reduces the volume of the plasma, but increases the volume of cells in the blood of intact animals Large doses diminish the blood flow through the kidneys and urine excretion (286, 287, 407) Small doses induce diuresis (337, 407), presumably by constriction of the efferent arterioles Smith (63, 368), because adrenalin reduced renal blood flow and urine formation without altering glomerular filtration, concluded that its action is always confined to constriction of the efferent arterioles If this inference, which has been challenged by Lamport (256, 257), is correct, the oliguria after large doses must result from excessive tubular reabsorption of water

As early as 1927 Baumann reported that the sodium and chloride in the serum of animals diminished after suprarenalectomy (25) and that the lives of such animals could be prolonged by injections of sodium salts (284) Loeb (271, 272) showed that the sodium of the serum of adrenalectomized animals and patients with Addison's disease was regularly low and that the symptoms of adrenal cortical insufficiency could be relieved or prevented for considerable periods by administration of large amounts of sodium chloride or sodium bicarbonate Removal of the adrenals is succeeded by a diuresis in which more sodium than water is sacrificed (201, 272) Swingle and associates (393) first called attention to hemoconcentration Fluid escapes from the blood stream, not only through the kidneys, but also through the capillary walls This transudation precedes the obvious symptoms and signs of circulatory collapse that characterize the fully developed syndrome of cortical failure (201, 393) This transudation must be due to circulatory failure For this the sodium deficit cannot be entirely responsible because cortical extracts will alleviate symptoms and signs of adrenal insufficiency before any reversal of the electrolyte disturbance or even the disposition of fluid in the body can be detected Adrenalectomized dogs are peculiarly susceptible to water intoxication (393) Patients in the crises of Addison's disease will develop edema if they are given parenteral fluids without

cortical extract (320) Reduction of the concentration of sodium in the extra cellular fluid causes the cells to imbibe water (309) Retention of potassium, which accumulates in the cells, should aggravate this tendency, but Harrison and Darrow (198) could detect no increases of cellular water in behalf of potassium suggesting that this element is retained in an osmotically inactive form

The chief effect of adrenalectomy upon the kidney is to diminish the reabsorption of sodium Chloride of serum usually falls proportionately, but in some cases of Addison's disease bicarbonate suffers instead (320) In the initial diuresis which immediately follows adrenalectomy more salt than water is lost. The excretion of water may signify that the general tendency to maintain the electrolyte osmotic pressure of the body fluids is not altogether abrogated The ability to reabsorb sodium is only impaired, not abolished Willson and Sunder man (440) found that the concentrations of sodium and chloride in the urine of a patient with Addison's disease did not increase to the usual degree after restric tion of fluid. Instead the sodium and chloride of the serum rose and the patient became dehydrated In advanced renal insufficiency with circulatory failure glomerular filtration diminishes and blood urea rises (396) Ultimately there may be complete anuria

Administration of cortical extracts completely reverses the disorders of adrenal insufficiency With free access to food, water and salt, serum electrolytes, body fluids, renal function and circulation become quite normal Sodium is retained while potassium is excreted If insufficient salt and water are given some body water is sacrificed with the potassium to restore the concentration of sodium in the extracellular fluids (200) Water diuresis may, therefore, attend both the onset and the termination of adrenal insufficiency

Adrenal cortical extracts and corticosterone have little effect on the metabolism of water and electrolytes in normal animals (130, 202, 203, 403 404) Acute retention of sodium after large doses of extract has been reported (202, 203, 404), but not sustained hypernatronemia. Conditions may be devised in which the effect of cortical extract would be evident, just as the antidiuretic action of the posterior pituitary extract is evident only after the administration of water The protective action of cortical extract against water intoxication (159, 393) may represent such a set up

Desoxycorticosterone has proved a valuable therapeutic substitute for the adrenal cortical hormone in the treatment of Addison's disease, although its action is confined almost entirely to the restraint of sodium wastage (386, 402, 405) It also has distinct effects on normal animals and, in large doses, has deleterious effects on adrenalectomized animals and patients with Addison's disease. Ferre beo et al (129) reported that excessive doses of desoxycorticosterone acetate, when given to adrenalectomized dogs or patients with Addison's disease, provoked retention of salt, sometimes sufficient to cause edema, together with hy pertension and signs and symptoms of congestive heart failure These disorders do not arise only from augmented tubular reabsorption of sodium, but also from wastage of potassium which leads to degeneration of the heart muscle (92, 93) They are aggravated by low potassium diets and can be mitigated or prevented

by administration of extra potassium (130, 329) In normal animals desoxycorticosterone provokes profuse diuresis with dilute urine (329)

If it be assumed that the action of desoxycorticosterone on the kidney is identical with that of the natural cortical hormone, all the phenomena that have been described could be explained on the hypothesis that the adrenal cortical hormone promotes reabsorption of sodium, while the posterior pituitary hormone promotes reabsorption of water It is not necessary to suppose that the actions of these two hormones are antithetical Since thirst is the natural response to accumulation of salt in the body, polydypsia should follow reabsorption of salt under the influence of desoxycorticosterone In rats (329) and in dogs (308) polyuria could not be induced with desoxycorticosterone unless salt was given The polyuria was preceded by an appreciable interval in which dogs retained water and gained weight (308) There was concomitant hemodilution (72, 308) The concentration of sodium in the serum rose (128, 308, 329), while its concentration in the urine fell (76, 308) Reduction of the urine volume by pituitrin did not cause the urinary concentration of chloride to rise as much as it does in true diabetes insipidus (308, 329) When dogs under the influence of desoxycorticosterone were deprived of water the polyuria subsided rapidly without the same degree of dehydration usually encountered in diabetes insipidus (308, 329) Cats with diabetes insipidus did not survive adrenalectomy as long as normal cats did (451, 452, 454) The negative sodium balance of adrenalectomy was not modified by diabetes insipidus, but serum sodium did not fall in cats with diabetes insipidus as it did in normal cats after removal of the suprarenal glands (454)

*The thyroid gland* Because it accelerates metabolism the thyroid hormone increases water of oxidation and losses of water through the skin and lungs The ratio of water lost by vaporization to heat production is abnormally large in hyperthyroidism (86, 237) In part, at least, this is referable to overactivity of the sweat glands, which may be diminished or prevented by atropine (237) In myxedema the proportion of heat eliminated by vaporization is unusually small (164)

In 1878 Ord (313) noticed the presence of a gelatinous fluid in the tissues of a patient who died of myxedema Boothby and associates (43) estimated that this peculiar edema fluid, which disappears when thyroid preparations are given, contains about 2 per cent of protein With its delivery sodium is lost, with only minimal quantities of potassium, indicating that the fluid and protein are deposited in the extracellular compartment (58) Elevated serum protein (98) and reduced blood volume (162, 397), which have been reported in myxedema, are logical consequences of the accumulation of protein in the interstitial spaces In hyperthyroidism the circulating blood volume tends to be abnormally large (42, 162)

Claims that the thyroid hormone has a specific diuretic action (124) have not been substantiated Mahoney and Sheehan (279) found that the polyuria produced in dogs by section of the hypophyseal stalk was inhibited by thyroidectomy and restored by thyroid therapy After hypophysectomy diabetes in-

sipidus can be induced by extracts of either the thyroid or the anterior lobe of the pituitary gland (246, 433), suggesting that the anterior lobe acts through the thyrotropic principle (35, 246) Ingram and Fisher (229) could not diminish the diabetes insipidus of cats by thyroidectomy, and White (432) was unable to induce diuresis in hypophysectomized rats with either anterior lobe or combination of anterior lobe and thyroid Swann and Johnson (391) diminished, but did not abolish, the polyuria of posthypophysectomized rats by thyroidectomy unless the animals were given salt This suggests that removal of the thyroid like removal of the anterior lobe of the hypophysis, alleviates the polyuria of diabetes insipidus only by diminishing the solutes requiring excretion In man Cutler (41, 85) has reported some improvement of diabetes insipidus after thyroidectomy, Findlay (134) has reported none, while Jonáš (235) claims beneficial effects from thyrotropic hormone

*The sexual hormones* Thorn and his associates claim that a large number of the steroid sexual hormones promote retention of sodium and water, but the significance of some of the changes reported is doubtful (406) In the case of a castrate male there was simultaneous retention of nitrogen, potassium and phosphorus (400) Scharpey-Schafer and Schnire (357) were unable to alter the urine volume of subjects with estradiol The incidence of menstrual edema in some women can be neither denied nor explained (16) There is suggestive evidence that progesterone may have an action resembling that of corticosterone (123, 401) The striking retention of fluid that accompanies tumefaction of the accessory sex organs of certain old world monkeys (254) is not relevant to humans This fluid has the characteristics of extracellular fluid (71)

*Pregnancy* Measurements of blood volume in pregnancy have, on the whole, yielded high values (99, 244, 346 399), the chief exceptions being data of Schoenholz (350) by the carbon monoxide method Thomson, Hershcimer et al (399), using the dye T-1824, found that the plasma volume rose progressively from early pregnancy to reach the astonishing figure of 65 per cent above normal at the end of the ninth month It then declined slowly to about 50 per cent above normal just before term and descended rapidly to normal in the first two weeks after delivery The volume of circulating blood cells rose to a lesser degree Others have reported smaller expansions These increases are far out of proportion to the comparatively small rises of oxygen consumption and blood flow Arterial blood pressure remains unchanged, venous blood pressure, except in the lower extremities, is not regularly elevated (56), disturbances of circulatory dynamics that might be anticipated from such a large addition to the circulation have not been discovered One is forced to wonder whether, for technical reasons, the dye method does not give excessive values

By means of thiocyanate Chesley (64) found that the extracellular fluid increased during normal pregnancy by an average of about 5 kgm of which 1.5 kgm could be accounted for by the uterus, membranes and fetus, an equal amount by expansion of the blood stream, leaving about 2 kgm as general enlargement of interstitial fluid About 1700 cc. of water were lost during delivery and about 2600 cc. in the first 6 days of the puerperium (65) Freyberg, Reekie

and Folsome (148) by metabolic measurements detected no increase of water during the last two months of pregnancy that could not be attributed to accessions of tissue The problem is further confused by the extreme variability of the water retained by different individuals Of 67 subjects studied by Chesley (64) over reasonably long intervals 6 gained from 0 to 50 ml of water per week, 14 gained over 200 Of another group about 17 per cent lost, rather than gained, extracellular water during late pregnancy Analyses of muscles of dogs (66) and of various tissues of guinea pigs (222) have revealed no evidences of abnormal quantities or distribution of water during pregnancy or after delivery

THE EFFECTS OF DIET, WATER AND SALTS *Starvation* is regularly attended by loss of a certain amount of water (33, 157, 442), part of which represents cellular water in which were dissolved protein and glycogen that are consumed (157, 442) Additional intracellular water and a larger quantity of extracellular water, with their salts, are also sacrificed This Gamble, Ross and Tisdall (157) ascribed to the effects of starvation acidosis This may play a part, especially in children who are peculiarly susceptible to ketosis, but in male adults Winkler, Danowski et al (442) were unable to prevent the diuresis by 100 grams of carbohydrate daily, enough to minimize ketosis Administration of water or salt solution does not prevent the initial dehydration (442) After 2 to 4 days an equilibrium is reached, after which water is lost in proportion to the tissue consumed (157)

*Malnutrition (protein starvation)* The origin of famine edema from protein deficiency was suggested (251, 292) before it was proved by experiment (149, 252) The edema results from reduction of serum albumin (50, 51, 423) It has been produced by low protein diets (149, 423) Serum albumin deficits have been observed in patients with a great variety of diseases and disorders accompanied by malnutrition (50, 51, 322) Transudation and depletion of serum albumin begin together at the very onset of protein starvation (122, 423), but edema at first is masked by loss of tissue, albumin deficiency by contraction of plasma volume As much as 5 to 6 kgm of fluid can accumulate in the interstitial spaces of an initially well developed adult male before edema is demonstrable by ordinary clinical methods (320) If an individual has some other disorder conducive to edema the effect of hypoalbuminemia is exaggerated In cardiac disease edema will appear or persist with a lesser degree of decompensation in malnourished patients with slight serum albumin deficits (316) The appearance of ascites and dependent edema in cirrhosis of the liver usually signifies that there is an associated hypoalbuminemia (57, 322)

*Excesses of water* After moderately large volumes of water (1 to 1 5 liters) diuresis does not begin until absorption is far advanced and long after there has been detectable reduction of the sodium and chloride concentrations in the serum, it reaches its peak only after absorption is completed (360, 361) The diuretic response, presumably effected by inhibition of posterior pituitary activity, is probably elicited by expansion of blood volume The initial fall of plasma electrolytes does not signify dilution, but the passage of salt into the alimentary canal It may even coincide with contraction of the extracellular

fluid because the loss of salt will cause cells to take up water The excretion of small or moderate increments of water is accomplished by reduction of reabsorption alone, large amounts increase glomerular filtration, probably because they swell the blood volume and raise glomerular capillary pressure (354, 355) Forcible administration of extremely large quantities of water provokes toxic symptoms, culminating in convulsions, collapse and death. These are referable not to the expansion of body fluids, but to the reduction of electrolyte osmotic pressure and consequent swelling of tissue cells They can be allayed by hypertonic saline in spite of the fact that this further swells the body fluids (190 344) On the other hand, concentrated urea solutions are of no benefit because the urea diffuses freely across the cell membranes and therefore does not correct the maldistribution of water (190)

*Water deprivation.* When animals are deprived of water insensible perspiration diminishes as dehydration becomes extreme (37, 91, 121, 442) Urine volume diminishes rapidly, presumably under the influence of the posterior pituitary hormone, until only enough is voided to eliminate in less than maximal concentration the solutes requiring excretion It is greatly influenced by the rate of protein metabolism (442) Conservation of water is accomplished by augmented reabsorption until the urine volume has reached a minimum and its concentration has become maximal, when glomerular filtration falls, probably because the reduction of blood volume and the inspissation of the blood lower the glomerular capillary pressure and raise the colloid osmotic pressure of the plasma (353) The blood urea rises. As dehydration advances water is lost out of proportion to sodium and chloride which are preferentially absorbed Besides water is lost in the insensible perspiration without salt, leaving only a fraction of the water to carry into the urine the salt which it held in the body The concentrations of sodium and chloride in the extracellular fluids rise and, by increasing the osmotic pressure, draw water from the cells The cells also yield a certain amount of potassium which permits the escape of further cellular water (37, 119, 120) Moderate dehydration is compatible with physical activity although the latter, by accelerating extrarenal dissipation of water, aggravates the dehydration Physical efficiency becomes impaired as dehydration progresses and loss of 10 per cent of the body water is totally disabling to men (442) In dogs death occurs, apparently from respiratory failure, when 25 per cent of the body water has been lost (91) Because it can concentrate urine more efficiently than man the dog can subsist on a high protein diet with less water The dog can subsist upon a diet of fish with no more water than can be obtained from the fish, man on a similar diet is forced to expend some of his own body water for the excretion of the nitrogen derived from the fish (442)

*Sodium deficiency* It is impossible by mere dietary restriction of salt seriously to deplete an animal of sodium, because the urinary output of sodium and chloride falls so rapidly Greenberg and Cuthbertson (175) found that rats could maintain chloride equilibrium, and even store chloride, on as little as one milligram per day The effect of withdrawing sodium by excessive sweating, vomiting, diarrhea, etc without replacing it, depends upon the amounts of water

simultaneously lost and the amounts taken to replace these losses When Darrow et al (95, 348) removed salt without water from the bodies of dogs by injecting glucose solutions intraperitoneally and subsequently withdrawing the fluid, the concentrations of sodium and chloride in the extracellular fluids fell and the volume of these fluids diminished because the cells swelled In such acute experiments there is no immediate effort of the kidneys to restore the composition of the body fluids A state resembling shock (348) ensues with almost complete anuria This may arise from reduction of the blood volume (300) associated with circulatory failure The animals evince little thirst (95, 165) If they are allowed access to water and are given salt-poor diets the sodium and chloride concentrations and the volume of the extracellular fluids remain low The kidneys will not preserve electrolyte osmotic pressure to the total neglect of the volume of the extracellular fluid

Loss of both water and salt in hypotonic solution, as in sweat, is comparable in its immediate effect to a primary loss of salt Sweating greatly accelerates the dehydration of water deprivation, including the increases of sodium and chloride in the serum If the losses of salt have been great, as they may be when heavy work is done at high temperatures, replacement of the lost fluid by drinking water may precipitate muscular and abdominal cramps (188, 306) and other symptoms of "water intoxication" Although the concentrations of sodium and chloride in the extracellular fluids are elevated, the total amounts of these ions in the body are low Replacement of the fluid by water alone, therefore, produces hypotonicity During actual work water may be taken to supply sweat In the intervals between activity, when sweating has ceased and water is taken to reconstitute the body fluids, salt is required (103, 395) This is less necessary after acclimatization is complete Salt taken during activity merely aggravates thirst and dehydration Ingestion of salt with insufficient water is highly undesirable because it drains more water into the urine when the kidneys are making every effort to conserve water Ladell (255) and others claim that men marooned at sea may benefit by supplementing scanty supplies of fresh water with sea water The evidence is not convincing that these supplements actually spare any body water (442) The concentration of salts in sea water approaches or exceeds the concentrating powers of the human kidney Drinking sea water alone is, therefore, disastrous Sea water enemas are equally dangerous (48, 143) Since the osmotic pressure of the contents of the gut can not be raised above the osmotic pressure of the body fluids, such enemas withdraw water into the gut and further raise the concentrations of sodium and chloride in the body

Because gastrointestinal and digestive secretions are isotonic their loss through vomiting, diarrhea and fistulae should deplete sodium and water in equivalent proportions Actually, since water continues to be dissipated in the insensible perspiration, if no food or fluids are given, more salt than water would be wasted If water is ingested a certain amount is absorbed, the remainder discharged in the vomitus, diarrhea or fistulous matter, carries with it salt that has been secreted into the water to make it isotonic These disorders, consequently,

usually produce a state of primary salt depletion (150, 156, 204) Since the volume of vomitus, especially in obstruction or irritation of the gastrointestinal tract, usually exceeds the volume of fluid ingested, the salt depletion is associated with a large deficit of water By prohibition of oral food and fluids the vomiting and diarrhea of mercury poisoning may be abolished (323), the vomiting of pyloric obstruction can be greatly reduced (142, 318) If decompression and lavage of the obstructed gut are practised (1, 2, 420) lavage should be performed, not with water, but with normal saline, to reduce secretory activity of the alimentary canal and the digestive glands to a minimum (83, 319) The discharge of fluid from high intestinal fistulae may be greatly diminished by giving only isotonic solutions of saline and glucose (320)

*Sodium excess* If the ratio of sodium salts to water is greater in the ingesta than in the extracellular fluid, the volume of the latter expands at the expense of the cells (95, 444) Diuresis ensues, its intensity depending upon the volume and salt concentration of the fluid given (196, 442) The diuresis is due chiefly to the limitation imposed upon tubular reabsorption of water by the osmotic effect of the salt Glomerular filtration is increased more by a given dose of hypertonic saline than by an equal amount of water (355) perhaps because the former, by drawing water from the cells, expands the extracellular fluid and the volume of the blood further

*Acidifying measures* If a strong acid which can not be utilized, for example a mineral acid, is given, it forms sodium salts in the body displacing bicarbonate The pH of the body fluids falls and, for reasons discussed above, the cells swell Since the kidneys can not eliminate the acid without some of the sodium with which it is combined, they respond, as they do to primary sodium deficiency, by sacrificing some water in the interests of the electrolyte osmotic pressure of the body fluids (14, 151, 158) Ammonium and calcium chloride have acidifying effects similar to that of hydrochloric acid The ammonia of ammonium chloride is converted to urea, leaving the chloride ion to form hydrochloric acid Calcium chloride is acidifying only when given by mouth, when a large part of the calcium is excreted in the feces while the chloride is absorbed (14, 151, 158)

*Alkalinizing measures* Administration of the hydroxide, carbonate or bicarbonate of sodium or sodium salts of organic acids that can be oxidized increases the sodium, bicarbonate and pH of the extracellular fluids The rise of pH causes the cells to shrink If sufficient water is available the excess of bicarbonate is eliminated by inhibition of its reabsorption in the tubules Sodium excess has, therefore, little diuretic effect, in pathologic states it may even conduce to edema (150, 156, 204) Spontaneous sodium excess has not been reported. Far commoner is replacement of chloride by bicarbonate, the condition encountered in patients who have vomited acid gastric contents (150, 156, 204) In such states there is invariably dehydration and sodium deficiency The sodium released by hydrochloric acid forms bicarbonate which is excreted by the kidneys to restore the normal anion pattern This leads to a sodium deficit that is met by sacrifice of water (150, 156)

*Salts other than sodium bicarbonate and chloride*, which distort the chemical

pattern of the body fluids are eliminated by the kidney with as much water as is required to permit their excretion in less than maximum concentration Because of the efficiency with which sulfate is excreted and its comparative physiological inactivity, its salts have a strong diuretic effect if they gain access to the body (38, 151, 351) They are, however, so slightly absorbed that, when given by mouth, they induce diarrhea, for diuretic purposes they must be given parenterally As a diuretic sodium sulfate is to be preferred (351), the magnesium salts, because of the toxic action of the magnesium ion, can not be given in large enough quantities (373, 447) The phosphate ion has other actions that contraindicate its general use as a diuretic The body seems peculiarly tolerant to nitrate, accepting it as a substitute for chloride (223) Keith (245) has recommended ammonium nitrate as an effective acidifying diuretic The chloride and organic salts of potassium are more diuretic than equivalent amounts of corresponding sodium salts, but can not be given in large enough quantities to have an important effect on urine volume

DIURETIC DRUGS AND CHEMICALS *Urea* is perhaps the blandest of all diuretics Diffusing freely through all the fluids of the body, without appreciable physiological activity, it does not even alter the distribution of water It can, therefore, be given in large quantities (30, 82, 277) It acts by limiting the reabsorption of water in the renal tubules

*Sugars*, when taken by mouth, have no significant effect on water metabolism except as they prevent ketosis, reduce protein catabolism and provide water of oxidation Isotonic glucose solution injected intravenously is equivalent from the standpoint of water metabolism to the injection of an equal amount of water because the glucose diffuses rapidly throughout the body and is consumed by the cells If the same solution is injected into a serous cavity or subcutaneously the water is absorbed more slowly than it would be from isotonic saline since salt diffuses into the glucose solution more rapidly than glucose diffuses out Initially such an injection may abstract water from the body at large (95) because, until equilibrium is established between the two media, the osmotic pressure is higher in the injected pool than in the rest of the body If hypertonic glucose solution is injected rapidly into a vein it draws fluid into the blood stream until the sugar has had time to diffuse out of the circulation This may elicit transient diuresis, but glucose exerts no important diuretic action unless it gives rise to glycosuria, because it is injected more rapidly than it can be utilized, because its utilization is impaired, or because its reabsorption in the renal tubules is inhibited by phlorizin Other monosaccharides have a similar effect if they are absorbed or injected more rapidly than they are consumed Disaccharides, with the exception of maltose, are not utilized if they enter the blood stream, but are rapidly and completely eliminated in the urine Because of its large molecular weight a given amount of disaccharide should command only half as much water as an equal weight of glucose This is partly offset by the fact that disaccharides escape reabsorption almost or quite completely and are excluded from cells from which they extract water by their osmotic effect

*The purine and mercurial diuretics* act directly upon the kidney (24, 34, 174)

There is some evidence that they inhibit primarily reabsorption of sodium chloride (270, 343), in which case the diuresis of water may be a secondary reaction. In keeping with this both the purine drugs (302) and the mercurials (270) in diabetes insipidus enable the kidneys to concentrate chloride in the urine, and therefore have an antidiuretic rather than a diuretic action in this condition According to Unna and Walterskirchen (409) pituitrin augments the diuretic effect of these drugs

POSTURE AND EXERCISE Stationary maintenance of the erect posture or a position in which the legs are immovably dependent leads to hemoconcentration with swelling of the lower extremities (398) because, under the influence of gravity venous stasis becomes great enough to cause transudation while lymphatic drainage is retarded (105) This edema can be prevented or eliminated by exercise of the lower extremities because muscular contraction facilitates the flow of blood and lymph (293)

Severe muscular exercise, by accelerating energy expenditure, increases water of oxidation and vaporization from lungs and skin (192), as well as blood flow Hemoconcentration, which has been reported (23, 342), is usually attributed to contraction of the circulating blood volume Ebert and Stead (108) found that the blue dye T-1824 could not be used to measure plasma volume in exercise because the inherent optical properties of serum are altered by exercise

During exercise muscles lose potassium (125, 180, 303), according to Miller and Darrow (303), with a proportional quantity of organic solid, probably glycogen

Severe exercise diminishes the rate of formation of urine (79, 111, 212, 345) This is not due only to the diversion of water to other excretory channels nor to hemoconcentration, since it is observed even when exercising subjects drink water (111, 212) Rydin and Verney (345) believe it is referable to action of the antidiuretic hormone of the pituitary If however, glomerular filtration is diminished, as Covian and Rehberg (79) claim there may be in addition a disturbance of renal circulation The excretion of chloride falls (111, 207) and the urea clearance diminishes more than the creatinine clearance does (79) Both the intensity and the duration of the oliguria vary directly with the severity of the exercise (79) and the oliguria persists for some time after exercise has ceased (207, 212)

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